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The growing role of immunotherapy and combination strategies in pancreatic and hepatobiliary cancers

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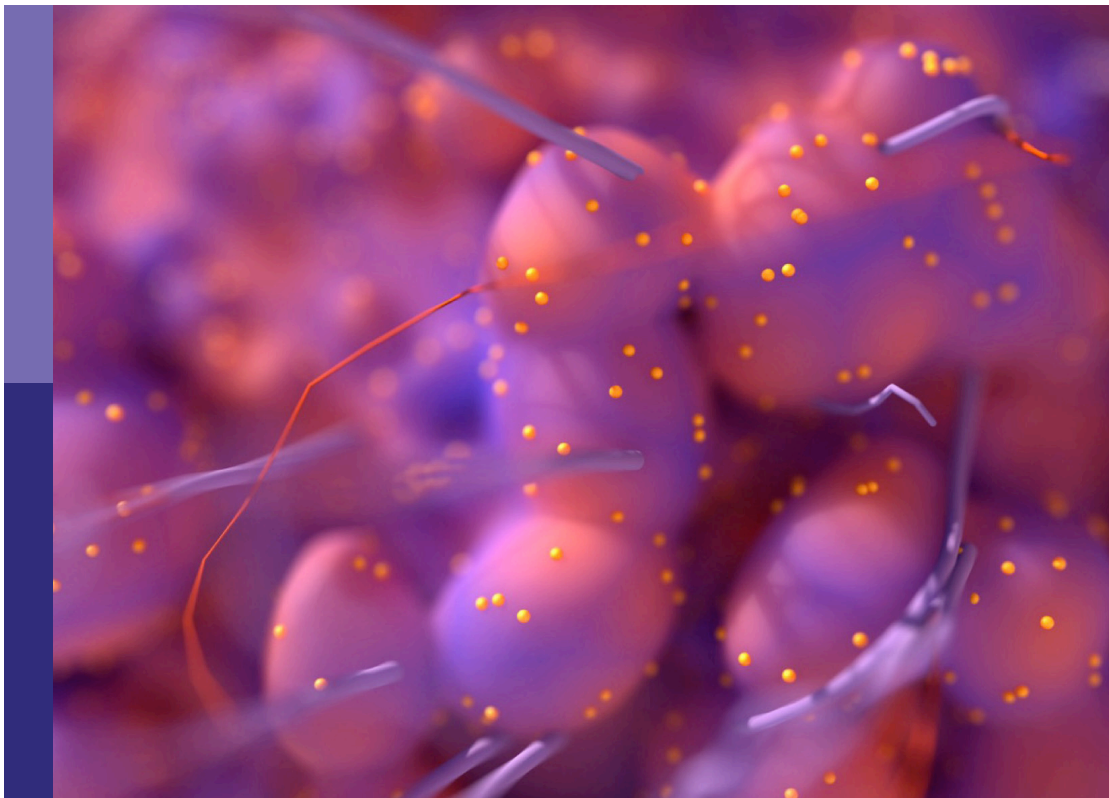

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The growing role of immunotherapy and combination strategies in pancreatic and hepatobiliary cancers

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Case report: Translational treatment of unresectable intrahepatic cholangiocarcinoma: Tislelizumab, Lenvatinib, and GEMOX in one case

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Background: Intrahepatic cholangiocellular carcinoma (ICC) is one of the most common invasive malignancies. Currently, ICC is treated with radical surgical resection. However, the majority of patients are diagnosed at an advanced stage, making surgery ineligible for them.

Case presentation: We present a case of advanced ICC, which could not undergo radical surgery due to tumor invasion of liver blood vessels. The gemcitabine and oxaliplatin (GEMOX) regimen combined with Tislelizumab immunotherapy and Lenvatinib targeted therapy for 8 cycles resulted in significant tumor shrinkage significantly and the vascular invasion disappeared. CA19−9 levels were reduced to normal levels. Partial remission and successful tumor transformation were achieved. The patient underwent a successful radical surgical resection, including cholecystectomy, resection of liver segments IV, V, and VIII, as well as a regional lymphatic dissection procedure, resulting in complete pathological remission.

Conclusion: Tumor-free surgical margins (R0) resection of patients with advanced ICC after combination of immune, targeted and chemotherapy is rare, and there are almost no cases of complete postoperative remission. The GEMOX regimen in combination with Tislelizumab and Lenvatinib has a good antitumor efficacy and safety profile, and may be a feasible and safe translational treatment option for advanced ICC.

KEYWORDS

advanced intrahepatic cholangiocarcinoma, chemotherapy, immunotherapy, targeted therapy, case report

Introduction

Cholangiocarcinoma (CCA) is an aggressive cancer of the biliary system and the second most common primary liver tumor. Its poor prognosis stems from its propensity for extensive metastasis, heightened resistance to pharmacological interventions, and the absence of efficacious therapeutic alternatives (1). Over the past decade, the occurrence rate of CCA surged from 0.67 cases per 100,000 individuals in 2007 to 1.40 cases per 100,000 individuals in 2016 (2). Intrahepatic cholangiocarcinoma (ICC), a subtype of CCA, originates from the epithelial cells of the intrahepatic bile ducts and has a 5-year overall survival rate of approximately 18% (3). The only treatment for ICC is surgical resection in order to achieve R0. However, due to its insidious onset and lack of typical clinical symptoms and biomarkers, it is often diagnosed only at advanced local or distant metastases. From 1983 to 2010, data from the SEER (Surveillance Epidemiology and End Results) database indicate that merely 15% of patients diagnosed with confirmed ICC were eligible for radical surgical resection (4). Data registered by the European Network for CCA Research (ENSCCA) between 2010 and 2019 validate these results (5). The emergence of enhanced chemotherapeutic, targeted, and immunological agents has ushered in a new era, where a multidisciplinary strategy comprising surgical interventions, systemic or localized therapies, and radiotherapy presents viable treatment avenues for individuals grappling with advanced ICC.

It wasn't until 2010 that gemcitabine/cisplatin (Gem/CDDP) chemotherapy was demonstrated to be an efficacious first-line regimen. To date, Gem/cisplatin (Gem/CDDP) chemotherapy is endorsed by the National Comprehensive Cancer Network (NCCN) as the primary treatment option for advanced ICC. However, the overall effect of chemotherapy is limited, with a low objective effectiveness rate (ORR) and susceptibility to drug resistance (6, 7). Programmed cell death protein 1 (PD-1) and its ligand, programmed cell death 1 ligand 1 (PD-L1), serve as immune checkpoint proteins, exerting a suppressive effect on immune responses. In the treatment landscape, inhibitors targeting PD-1/PD-L1 have exhibited encouraging outcomes across diverse cancer types (8). As of now, PD-1 inhibitors have not received approval for first-line systemic therapy in biliary tract cancers, except for Pembrolizumab, which is indicated for mismatch repair-deficient (dMMR) or high microsatellite instability (MSI) tumors. Numerous investigations have assessed the therapeutic effectiveness of PD-1 inhibitors when combined with chemotherapy for advanced biliary tract cancer (9–11).The combination of PD-1 inhibitors with chemotherapy significantly improved the median OS in patients with advanced ICC compared to PD-1 inhibitors alone and chemotherapy alone (10). Lenvatinib, characterized as a multi-targeted tyrosine kinase inhibitor (TKI), has gained approval for managing unresectable hepatocellular carcinoma (HCC). The combination of Lenvatinib and Immune checkpoint inhibitors (ICIs) has yielded positive results and offers a broader perspective for HCC. In terms of ICC, the phase II clinical trial (NCT02579616) used Lenvatinib as a single agent in the treatment of unresectable biliary tract cancer, and the results showed promising antitumor activity (12). Based on the drug safety of the combination of Lenvatinib and ICIs, several recent studies have used the combination of Lenvatinib and ICIs in the treatment of advanced ICC. Xie et al. conducted an evaluation on the effectiveness of combining Lenvatinib with a PD-1 inhibitor in a cohort of 40 patients diagnosed with intrahepatic cholangiocarcinoma (ICC), who had experienced treatment failure with chemotherapy for advanced disease. Their findings demonstrated that the combination of Lenvatinib and PD-1 inhibitors exhibited notable efficacy in addressing advanced ICC following chemotherapy resistance (13).

Many studies have demonstrated the ability of chemotherapy combined with targeted and immunotherapy to alleviate advanced biliary tract cancer (BTC). However, there are few cases of complete remission after resection of advanced ICC conversion therapy (14). In this report, we present the case of a patient diagnosed with unresectable advanced intrahepatic cholangiocarcinoma (ICC) who achieved complete postoperative remission without recurrence following successful surgical R0 resection. The patient underwent the GEMOX regimen in combination with PD-1 inhibitors Tislelizumab and Lenvatinib as first-line treatment. This case offers valuable insights and guidance for the clinical management of advanced ICC.

Case presentation

On 3 September 2022, a 51-year-old Chinese woman presented to our hospital with "20 days of jaundice in the whites of the skin and eyes". On the 10th day of her jaundice, she underwent percutaneous transhepatic cholangial drainage and ultrasound-guided biopsy of the liver tumor on 23 August 2022 at an outside hospital. Pathological examination (Figure 1F) showed intrahepatic cholangiocarcinoma. Her occupation is worker, and her education level is primary school. There was a past history of breast cancer 12 years ago, which was cured. The patient's medical history revealed no past or present alcohol consumption or hepatitis B infection. There were no tumors in her family. Physical examination of her revealed generalized jaundice of the skin, mucous membranes and sclera. She exhibited epigastric tenderness without rebound tenderness, lacked any significant abdominal mass, and showed no notable enlargement of the subclavian lymph nodes. Laboratory investigations revealed significant elevation of glycan chain antigen 199 (CA199) (589.7 KU/L, normal range 0–25 KU/L), glycan chain antigen 125 (CA125 (72.3 KU/L, normal range 0–23 KU/L), carcinoembryonic antigen (CEA) (25.3 ug/L, normal range 0–5 ug/L), alanine aminotransferase (91 U/L, normal range 7–40 U/L), aspartate aminotransferase (52 U/L, normal range 13–35 U/L), total bilirubin (93.5 μmol/L, normal range 0–21 μmol/L), elevated direct bilirubin (51.8 μmol/L, normal range 0–4 μmol/L, elevated indirect bilirubin level (41.7 μmol/L, normal range 0–21 μmol/L). Alpha-fetoprotein (AFP) and glycoconjugate antigen 125 (CA125) levels were in the normal range. Abdominal plain + enhanced CT showed hepatoportal occupancy, consideration of intrahepatic bile duct dilatation secondary to cholangiocellular malignancy. Abdominal MRCP showed multiple abnormal signals in the liver, marked dilatation of the intrahepatic bile ducts, and partial truncation of the bile ducts in

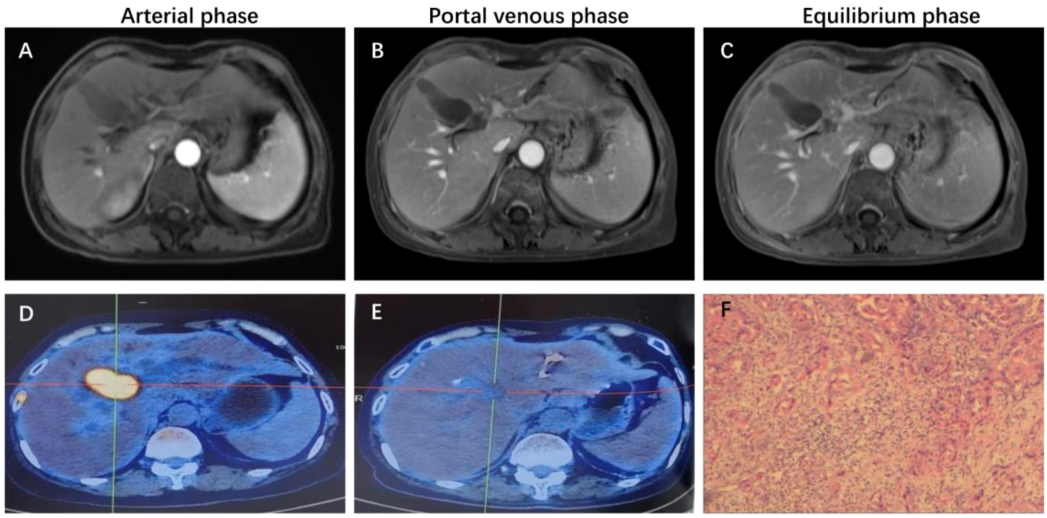

FIGURE 1
Representative MRI imaging and PET-CT maps at the end of 8 cycles of combination therapy. **(A-C)** At the end of the combined treatment, the liver enhanced MRI axial T1 weighted images of artery, portal vein and balance phase showed no obvious enhancement of the tumor. **(D)** Increased 18F-FDG metabolism of the mass is seen on PET-CT of the liver tumor before combination therapy. **(E)** significant decrease in 18F-FDG metabolism in the visible mass of liver tumor PET-CT at the end of combination therapy. **(F)** H&E staining results of liver tumor biopsy guided by ultrasound.

the portal region. CTA of the hepatic artery, CTV of the portal vein, CTV of the hepatic vein, and CTV of the inferior vena cava showed intrahepatic cholangiocarcinoma with possible hepatoportal metastasis, with the lesion locally adhering to the right branch of the portal vein and the right hepatic artery, and the intrahepatic bile ducts were dilated. Based on these findings, we diagnosed ICC with intrahepatic vascular invasion. As per the 8th edition of the American Joint Committee on Cancer (AJCC) Cancer Staging Manual, the clinical TNM stage was identified as T2N0M0 (stage II).

Treatment

This patient was unable to undergo radical resection due to tumor invasion of intrahepatic vessels. After a multidisciplinary consultation, on 15 September 2022, the patient was initiated to receive GEMOX chemotherapy (gemcitabine 1000 mg/m2, iv, d1, d8/3w combined with oxaliplatin 125 mg/m2, iv, d2/3w) and anti-PD-1 immunotherapy (Tislelizumab 200 mg, iv, q3w) combined with targeted therapy (Lenvatinib 8 mg, po, qd) as a treatment regimen. Adverse events that occurred during treatment included decreased appetite, nausea, peripheral sensory neuropathy, thrombocytopenia, neutropenia and drug-induced liver injury. Recombinant human granulocyte stimulating factor was used to correct neutropenia in patients. Severe thrombocytopenia was ameliorated by platelet transfusion and recombinant human interleukin-11. We administered N-acetylcysteine (NAC) and Polyene Phosphatidylcholine injections to patients to improve drug-induced liver damage. In response to the nausea and vomiting that occurred during the treatment process, we provided patients with Palonosetron and Dexamethasone injections to improve symptoms. All adverse events related to immunological and chemotherapy treatments were effectively managed and resolved following the completion of treatment. After 1 cycle of conversion therapy, two nodules were found in the hilar region, the one near the hilar area was smaller than before (Figures 2A, B), and the other nodule was significantly altered in morphology and had a “pipe-like” shape (Figures 2D, E). After 3 cycles of conversion therapy, one nodule near the porta hepatis continued to shrink compared with the previous one (Figure 2C), and the other nodule showed no significant change (Figure 2F). After 8 cycles of treatment, abdominal CT showed that one nodule near the porta hepatis continued to shrink compared with the previous one, the other nodule showed no significant change, and the tumor showed no significant enhancement on enhanced magnetic resonance (Figures 1A–C), no tumor invasion of blood vessels was found. At the end of 8 cycles of combination therapy, the metabolism of 18F-FDG in the liver tumor decreased significantly by PET-CT (Figures 1D, E), suggesting that the treatment was effective. CA19–9 levels (Figure 3A) and CEA levels (Figure 3B) showed a continuous decreasing trend during the treatment. Tumor efficacy was evaluated according to the mRECIST criteria and was judged as partial remission (PR). The changes of total bilirubin during the whole treatment process are shown in Figure 3C. The patient concluded the final cycle of conversion therapy on February 19, 2023. One month after discontinuation of combination therapy, the patient’s routine hematology showed mild anemia, with no significant abnormalities in coagulation or renal function test results.CA199, CA125, CEA, AFP and CA125 were in the normal range. Alanine aminotransferase (79 U/L, normal range 7–40 U/L), aspartate aminotransferase (60 U/L, normal range 13–35 U/L), total bilirubin (13.9 μmol/L, normal range 0–21 μmol/L), elevated direct bilirubin (5.3 μmol/L, normal range 0–4 μmol/L, elevated indirect bilirubin level (8.6 μmol/L, normal range 0–21 μmol/L). On 26 March 2023, the patient underwent hepatic resection of liver segments IV, V and VIII + cholecystectomy + regional lymph node dissection.

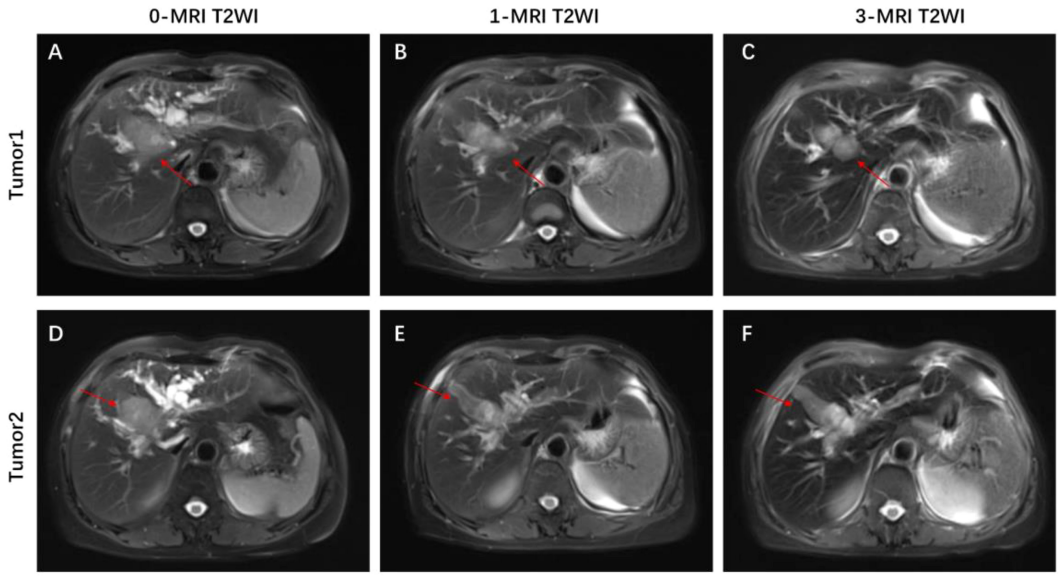

FIGURE 2
Representative MRI images showing changes in the tumor before and after combination therapy. **(A, D)** axial T2-weighted MRI images of the liver before combination therapy, the tumor consists of two parts. **(B, E)** Axial T2-weighted MRI image of the liver at 1 cycle of combination therapy, with a shrinkage of the mass near the hilar region and a change in the morphology of the other mass. **(C, F)** Axial T2-weighted MIR image of the liver at 3 cycles of combined treatment, with significant shrinkage of the mass near the hilar region.

Operative findings

A reverse L-shaped incision was made in the right upper abdominal region to perform the dissection. The surface of the liver was smooth, and ultrasound examination revealed two nodules in the porta hepatis with diameters of about 3.0 and 1.8 cm. Enlarged lymph nodes with hard texture could be found in the hepatoduodenal ligament, posterior duodenum, adjacent to the common hepatic artery, and hepatogastric hiatus. The posterior aspect of the pancreatic head, liver, pelvis, small intestine, colon, and mesentery exhibited no abnormalities upon examination. The gallbladder was firstly removed intact. Subsequently, enlarged lymph nodes, fat and lymphatic tissue in the gallbladder triangle, hepatoduodenal ligament, and adjacent to the common hepatic artery were removed, and the hepatoduodenal ligament was skeletonized and cleared. Lymph nodes in the hepatogastric space were cleared, followed by complete hemostasis. To ensure a wide (>1 cm) resection margin, Intraoperative ultrasound was employed to determine the spatial relationship between the tumor and the hilar and intrahepatic vessels, leading us to conclude that anatomical hepatectomy was viable. Therefore, segment IV, V and VIII hepatectomy was performed. Hepatic parenchyma resection was performed with ultrasonic knife. After hepatic tumor resection, suture ligation or electrocautery was used to control residual bleeding sites. The surgical procedure proceeded smoothly, lasting 300 minutes, with an estimated blood loss of 100 ml. The surgical resection specimen is displayed in Figures 4A, B. The tumor was seen to consist of two parts, measuring approximately 6.5 x 2.5 x 2.5 cm and 2.6 x 2 x 0.8 cm, and was greyish-white in color.

Prognosis and follow-up

The postoperative pathological examination using hematoxylin and eosin (HE) staining (Figure 4C) revealed extensive sampling (23 pieces) of the original tumor bed area, all of which showed tumorous extensive necrosis, peri necrotic fibrosis with inflammatory cell infiltration, consistent with post-treatment changes, and no active tumor components were seen. The tumor measured 6.6x2.5x2.5 cm with peritumoral invasion and no significant cancer thrombus was seen in the vessels. The surgical margins were clear. There were no metastases in the lymph nodes of groups 8 and 12A, 12B, 12P (0/3) and no metastases in the left gastric lymph node (0/7). The gallbladder showed chronic cholecystitis (Figure 4D).

This patient was discharged 19 days postoperatively (14 April 2023) after an uneventful postoperative course. After surgery, GEMOX chemotherapy (gemcitabine 1000 mg/m2, iv, d1, d8/3w in combination with oxaliplatin 125 mg/m2, iv, d2/3w) and anti-PD-1 immunotherapy (Tislelizumab 200 mg, iv, q3w) in combination with targeted therapy (Lenvatinib 8 mg, orally, qd) were continued on 4 July 2023 as a treatment regimen and continued until disease progression. Six cycles of combined chemotherapy were performed after operation. During the first-year post-surgery, follow-up visits were conducted monthly, encompassing physical examinations, routine blood tests, blood biochemistry analyses, tumor marker assessments, and abdominal MRI was reviewed every three months. The patient's timeline, including initial diagnosis, combination therapy, radical surgery, and follow-up, is depicted in Figure 3D. As of March 2024, the patient shows no signs of local recurrence or distant metastasis of the tumor (Figure 3E).

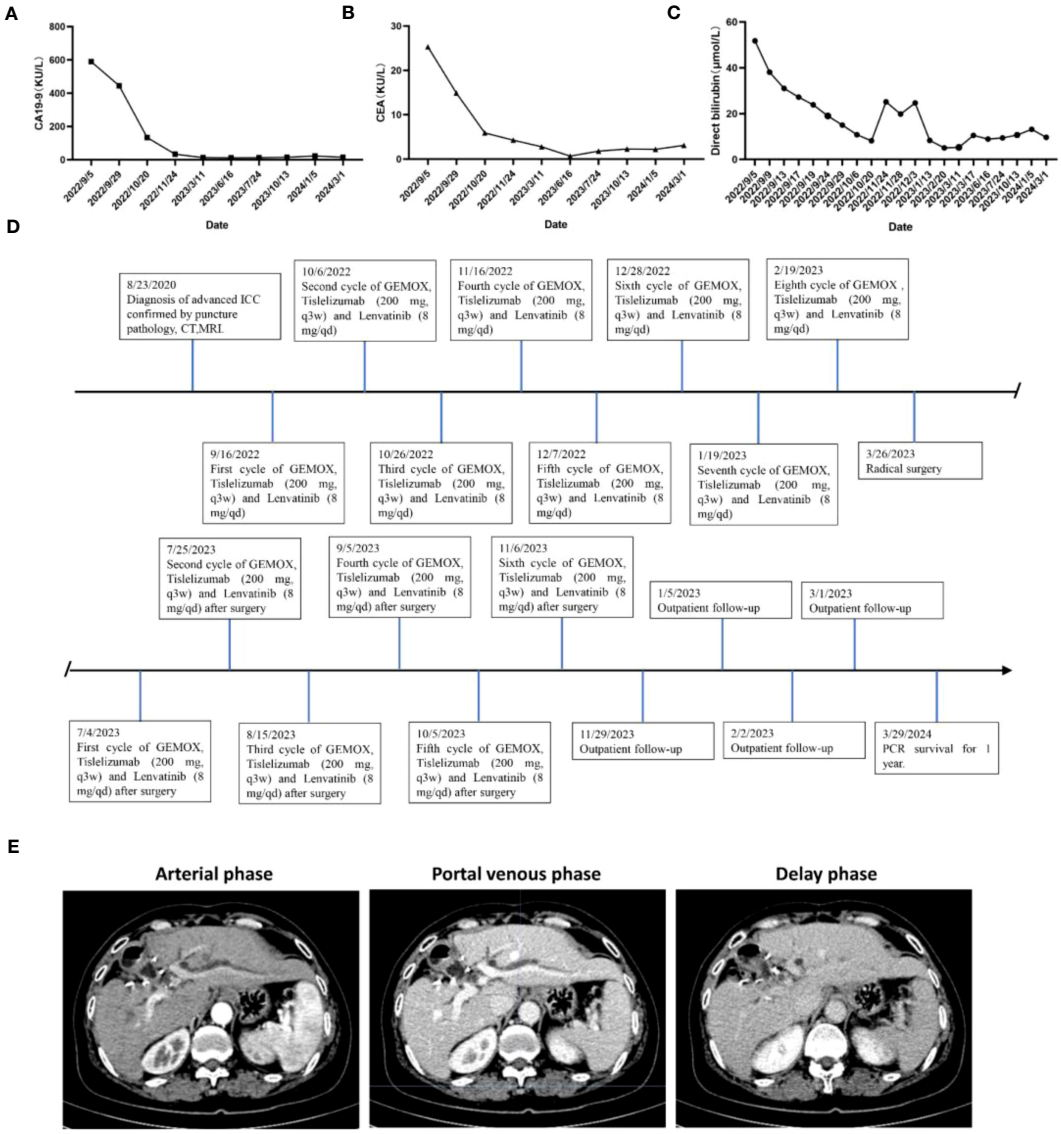

FIGURE 3
Changes in **(A)** CA19−9, **(B)** CEA expression levels and levels of **(C)** total bilirubin during combination therapy for advanced ICC. **(D)** Timeline of initial diagnosis, utilization of combination therapy, radical surgery, adjuvant therapy and subsequent follow-up. **(E)** Enhanced CT scan of liver 1 year after operation.

Discussion

Cholangiocarcinoma presents as an aggressive tumor with a bleak prognosis, posing significant challenges in treatment strategies. Studies have shown that the age-standardized mortality rate for overall patients with ICC has increased by 36.5% in men and 36.2% in women in 13 European Union countries, with similar increases in the United States and Australia (15). The NCCN guidelines categorize intrahepatic cholangiocarcinoma (ICC) into resectable, unresectable, and metastatic disease subtypes, with therapeutic considerations tailored accordingly (16). Surgery stands as the cornerstone treatment for intrahepatic cholangiocarcinoma (ICC), aiming to attain R0 resection while ensuring preservation of sufficient function in the future liver remnant (FLR). Nonetheless, only a minority of individuals with early-stage disease, approximately 35%, meet the criteria for surgical resection therapy (17). In our case, the tumor invaded the intrahepatic blood vessels and could not undergo radical surgery. For patients with advanced inoperable ICC, several therapeutic options are available, including systemic and targeted therapy, locoregional therapy, and radiotherapy. The NCCN guidelines advocate for cisplatin/gemcitabine (Gem/CDDP) combination chemotherapy as the preferred first-line treatment option. The ABC-02 trial, published in 2010, demonstrated that patients receiving Gem/CDDP chemotherapy for advanced biliary tract cancer achieved a median overall survival (OS) of 11.7 months (18). Oxaliplatin in combination with gemcitabine (GEMOX) is another common treatment regimen. The study by Fiteni F et al. included 771 and 699 patients with advanced ICC treated with Gem/CDDP and GEMOX, and showed that the weighted median survival for the treatment in the Gem/CDDP group and the GEMOX group

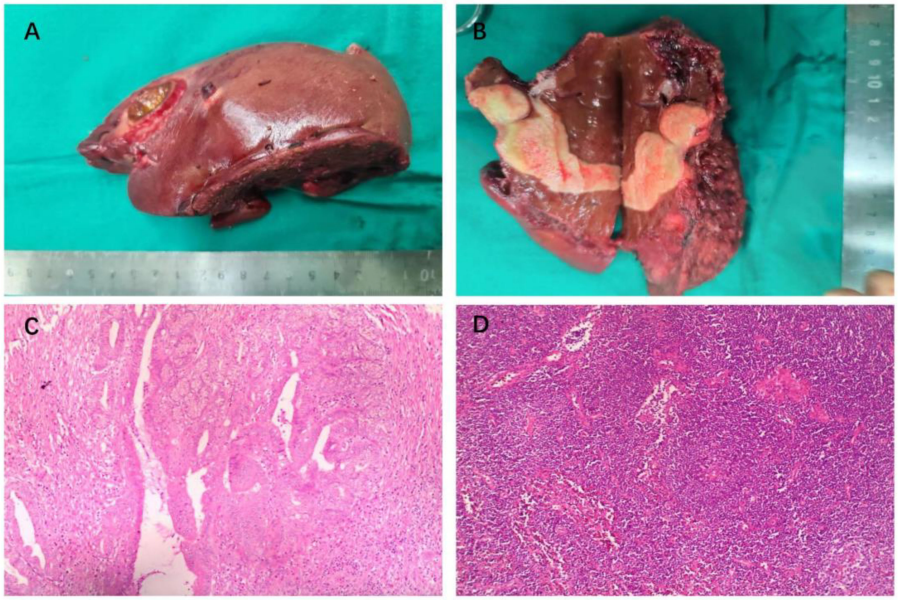

FIGURE 4
Intraoperative gross pathology specimens and HE staining of radical resection in a patient with advanced ICC. **(A)** Resection specimens of liver segments IV, V and VIII, measuring about 14x7x6 cm. **(B)** The visible tumor consists of two parts, measuring about 6.5 x 2.5 x 2.5 cm and 2.6 x 2 x 0.8 cm. **(C)** H&E staining shows extensive necrosis (>95%) of the tumor tissue, with predominantly fibrotic and inflammatory cellular infiltration, and no tumor-active components were seen. **(D)** H&E staining showed chronic inflammation of gallbladder.

was 9.7 months and 9.5 months, respectively. Survival outcomes were comparable between the two groups; however, Gem/CDDP chemotherapy was notably linked to a higher incidence of grade 3 and 4 adverse events, including malaise, diarrhea, hepatotoxicity, and hematotoxicity (19). In numerous cancer institutions, GEMOX chemotherapy is frequently favored as the first-line chemotherapy regimen. Zhu et al. included 53 ICC patients who received PD-1 inhibitor combined with lenvatinib and Gemox chemotherapy for a retrospective study, and all patients experienced grade 3 or 4 adverse events, including fatigue and bone marrow suppression (20). Shi et al.'s Phase II clinical study included 30 advanced stage ICC patients receiving gemcitabine and oxaliplatin combined with toripalimab and lenvatinib treatment. As a result, 56.7% of patients experienced grade 3 or higher adverse events, mainly bone marrow suppression (21). In our case, the patient also experienced adverse events of ≥ 3 levels, such as neutropenia and leukopenia, consistent with previous studies. In addition, our patient experienced liver toxicity, with alanine aminotransferase reaching a maximum of 246U/L (normal range of 7–40 U/L). Through appropriate drug intervention, these adverse events were effectively controlled and improved without affecting the treatment plan.

Median overall survival with chemotherapy alone still did not exceed 1 year, an unsatisfactory result. Cancer cells often exploit PD-1 signaling to evade immune surveillance (22). ICIs exert their anti-tumor effects by targeting cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), PD-1 and PD-L1 (23). Chemotherapy may improve immunotherapeutic efficacy by destroying tumor tissue, overcoming immune rejection, and facilitating cross-presentation of tumor antigens (24–26). On 21 December 2022, Europe (EU) approved Durvalumab plus Gem/CDDP chemotherapy as a first-line treatment regimen for patients with advanced BTC. A phase II clinical trial conducted by Chen, Xinni et al. showcased that Camrelizumab, when combined with FOLFOX4 or GEMOX as first-line treatment, exhibited efficacy and tolerability in Chinese patients with advanced BTC. The study reported median progression-free survival of 5.3 months (95% CI = 3.7–5.7) and median overall survival of 12.4 months (95% CI = 8.9–16.1) (27). In a study by Ueno et al., it was suggested that patients with unresectable or recurrent biliary tract cancer (BTC) who underwent their initial chemotherapy treatment with Nivolumab in combination with Gem/CDDP chemotherapy achieved a median progression-free survival of 4.2 months (90% CI 2.8–5.6) and a median overall survival of 15.4 months (90% CI 11.8 - not estimable) (28). The aforementioned studies demonstrate favorable anti-tumor efficacy and manageable safety profiles in patients with advanced unresectable or metastatic BTC.

In recent years, significant advancements have been achieved in comprehending the molecular biology of ICC and in the development of pertinent targeted therapies. Lenvatinib, a multi-targeted tyrosine kinase inhibitor, finds utility as monotherapy or in combination with other anticancer agents in the treatment of radioiodine-refractory differentiated thyroid cancer, hepatocellular carcinoma, renal cell carcinoma, and endometrial cancer (29). Combining ICIs with TKIs is firmly supported by a robust biological rationale (30, 31). Lenvatinib may eliminate ICC cells through immunogenic cell death, reduce the number of cells targeted and destroyed by immune cells, and improve the efficacy of immunotherapy (32). Faiz et al.'s study showed that PD-1/PD-L1 is overexpressed in ICC (33), while Tian et al. further confirmed that the high expression of PD-1 in ICC tissue is associated with

TABLE 1 Published trials and case reports of ICI combined with Targeted therapies.

Author	Country	Phase	Treatment Arm(s)	Patients (n=)	ORR (%)	PFS (Months)	OS (Months)	References
GuoMing Shi	China	II	Toripalimab+ Lenvatinib+GEMOX	30	80	10.2	22.5	(21)
Zhou Jian	China	II	Toripalimab+ Lenvatinib+GEMOX	30	80	10	–	(36)
Chengpei Zhu	China	II	ICIs+ Lenvatinib+GEMOX	57	43.9	9.27	13.4	(37)
H Li	China	II	Tislelizumab lenvatinib+GEMOX	25	56	–	–	(38)
Wei Zhang	China	–	Tislelizumab lenvatinib+GP	1	–	–	–	(14)
Zhuochao Zhang	China	–	Toripalimab +Pemigatinib + GEMOX	1	–	–	–	(39)

better OS (34), possibly due to PD-L1 inhibiting macrophage activity and survival rate, while PD-L1 antibodies can enhance the ability of macrophages to secrete inflammatory cytokines while promoting T cell proliferation and activation (35). Zhou et al. conducted a phase II clinical trial comprising 30 patients diagnosed with pathologically confirmed advanced intrahepatic cholangiocarcinoma (ICC). These patients were administered Lenvatinib in conjunction with GEMOX chemotherapy and the anti-PD-1 antibody Toripalimab as first-line therapy. The study reported a median progression-free survival (PFS) of 10.0 months, and the median overall survival (OS) was not reached. The objective response rate (ORR) was recorded at 80% (36). In a phase II clinical trial conducted by Zhu et al., 57 patients diagnosed with advanced biliary tract cancer (BTC) were treated with Lenvatinib in combination with a PD-1/PD-L1 inhibitor and GEMOX chemotherapy. The study reported a median overall survival of 13.4 months (95% CI: 10.0-NA) and a median progression-free survival of 9.27 months (95% CI: 7.1–11.6) (37). Table 1 shows the research results of ICIs combined with immunotherapy and chemotherapy drugs for the treatment of advanced BTC. These studies suggest that the integration of immunotherapy with targeted therapy and systemic chemotherapy holds promise in providing therapeutic benefits for patients with advanced BTC achieve relatively good ORR, and further large-scale clinical trials are still warranted to validate the efficacy of the combination regimen. In the study presented in Table 1, the number of patients who achieved R0 surgical resection following conversion therapy was limited. Surgical resection is not the ultimate goal of conversion therapy; rather, the ultimate goal is for patients to achieve long-term survival and a high quality of life. Therefore, in terms of selecting the timing of surgery, we believe that if the treatment regimen is effective, conversion therapy should be allowed to reach a certain depth before surgery. Efforts should be made to achieve imaging complete response (CR) or more than 90% tumor necrosis before surgery. For tumors that do not shrink after two reassessments or if AFP levels do not decrease, timely surgical intervention should be performed. This approach aims to prevent postoperative tumor recurrence or metastasis due to insufficient depth of conversion therapy. During the treatment period, we rechecked blood tumor markers every 21 days and conducted liver MRI examinations every 2 months to evaluate changes in tumor size and morphology, thereby assessing the efficacy of the conversion therapy. In our report, the combination therapy had significant anti-tumor activity. After 8 cycles of treatment, the initially unresectable ICC was significantly smaller than before, and pathologically the tumor cells were completely necrotic and the structure disappeared. Patients were offered R0 resection with normalized tumor marker levels and no recurrence or distant metastases. If the tumor continues to grow during treatment, MDT discussion should be organized in time to change the treatment plan for patients to obtain the maximum benefit. Despite experiencing depression at the initial diagnosis of ICC, the patient ultimately found satisfaction with the treatment and demonstrated good compliance through our comprehensive therapeutic approach.

This study has limitations. A single case is not enough to prove the universality and effectiveness of this treatment scheme in all patients with advanced ICC. To validate the efficacy of this combination therapy, conducting larger-scale clinical trials is imperative.

Conclusion

In conclusion, the findings of the study indicate that immune and targeted combination chemotherapy exhibits favorable anti-tumor efficacy and safety in certain patients with advanced intrahepatic cholangiocarcinoma (ICC). This approach holds promise as a potential, feasible, and safe translational treatment strategy for advanced ICC. However, further large-scale clinical trials are necessary to validate these findings definitively.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving humans were approved by the ethics committee of Wenzhou Central Hospital. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study. Written informed consent was

obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

HZ: Writing – original draft, Writing – review & editing. H-bY: Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Evaluating the benefits of adjuvant chemotherapy in patients with pancreatic cancer undergoing radical pancreatectomy after neoadjuvant therapy—a systematic review and meta-analysis

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Background: More and more patients with pancreatic cancer (PC) received neoadjuvant therapy (NAT) and then underwent radical pancreatectomy. However, the benefit of adjuvant chemotherapy (AC) for these patients is still controversial. This study is designed to determine the benefits of postoperative AC for patients with PC undergoing NAT and radical resection.

Methods: We conducted a comprehensive search of the PubMed, Embase, Web of Science, and Cochrane Library databases, covering the period from their inception until 10 September 2023. Our analysis focused on the assessment of overall survival (OS) and recurrence-free survival (RFS) through meta-analysis. The fixed-effects model and the random-effects model were used to process the data. Hazard ratios (HRs) and 95% confidence intervals (95% CIs) were employed to determine the necessary of administering AC for patients with PC who have undergone NAT and radical resection. We retrieved 3,063 search results, of which 3,048 were excluded because of duplication or after applying our inclusion and exclusion criteria.

Results: A total of 15 studies with 21,113 patients (7,794 patients in the AC group and 13,319 in the non-AC group) were included, all of which reported OS, and three studies reported disease-free survival (DFS)/tumor-specific survival (CSS)/RFS. The final results showed that AC significantly improved OS and DFS/CSS/RFS in patients with PC who underwent pancreatectomy after NAT [OS: HR = 0.80, 95% CI (0.75~0.86), P < 0.00001, I^2 = 48%; DFS/CSS/RFS: HR = 0.53, 95% CI (0.41~0.69), P < 0.00001, I^2 = 0%]. Furthermore, we performed subgroup analyses and demonstrated that AC provided a significant survival benefit for patients with PC after NAT and resection regardless of the tumor size [<2-cm subgroup: HR = 0.72, 95% CI (0.5~0.94), P = 0.01; ≥2-cm subgroup: HR = 0.79,

95% CI (0.65~0.96), P = 0.02] and the margin status [R0 subgroup: HR = 0.83, 95% CI (0.77~0.88), P < 0.00001; R2 subgroup: HR = 0.75, 95% CI (0.61~0.92), P = 0.007]. AC also benefited the patients with a stage N0 [HR = 0.79, 95% CI (0.74~0.84), P < 0.00001], N1 [HR = 0.78, 95% CI (0.72~0.85), P < 0.00001], or poorly/undifferentiated tumor [HR = 0.76, 95% CI (0.66~0.87), P < 0.0001] in survival but not in patients with a stage N2 [HR = 0.69, 95% CI (0.43~1.09), P = 0.11] or well/moderately differentiated tumor [HR = 0.97, 95% CI (0.66~1.42), P = 0.87].

Conclusions: Although AC showed survival benefit for patients with PC undergoing radical pancreatectomy after NAT, we still need to consider the lymph node stage and the degree of differentiation of the tumor when we gave AC to a patient. High-quality prospective randomized controlled studies are required to well disclose the value of AC in patients with PC undergoing radical pancreatectomy after NAT.

Systematic review registration: https://www.crd.york.ac.uk/prospero/ PROSPERO, identifier CRD42023461365.

KEYWORDS

adjuvant chemotherapy, neoadjuvant therapy, pancreatic ductal adenocarcinoma, overall survival, disease-free survival, TNM-staging

1 Introduction

Pancreatic cancer (PC) is one of the most aggressive and lethal malignancies with a 5-year overall survival (OS) of less than 10% (1). Given the high mortality and increasing incidence every year, PC is projected to become the second leading cause of cancer-related death by 2030 (2). Currently, surgical resection is still the only radical treatment for PC. However, the effect of operation is not good, the 5-year OS is as low as 20%, whereas about 75% of patients will experience tumor recurrence within 2 years (3–5). Systemic adjuvant chemotherapy (AC) plays a crucial role in treatment of patients underwent radical resection in considering that PC is a systemic disease (6). Disappointingly, the improvement of systemic AC on the survival of patents after radical resection is still limited (7).

Neoadjuvant therapy (NAT) has been become the important treatment for patients with localized PC (8), which downstages the primary tumor, increases the feasibility of R0 resection, eliminates micrometastasis, and identifies aggressive tumors to avoid futile surgery (9, 10). Moreover, nearly half of the patients are unable to receive AC due to surgical complications after operation (11), and preoperative chemoradiotherapy successfully overcomes this situation. The prolonged disease-free survival (DFS) and OS also confirms the advantages of NAT in patients with high risk resectable and borderline resectable PC (12, 13).

However, the necessity and benefits of AC in patients with PC underwent pancreatectomy after NAT remains controversial. Sugawara et al. found that patients with PC received AC after NAT, and resection had significantly better survival benefits than those did not receive AC (14). In contrast, van Roessel's team indicated that additional postoperative therapy may not provide an additional survival benefit, except for patients with pathologically confirmed lymph node-positive PC (15).

To well disclose the value of AC in patients with PC underwent pancreatectomy after NAT, we conduct a systematic review and meta-analysis, the effects of AC on survival and the potential benefit subgroups will be reported.

2 Materials and methods

2.1 Literature search strategy and selection criteria

The PubMed, Embase, Web of Science, and Cochrane Library databases from inception through 10 September 2023 were searched for literature published in English comparing the need for postoperative AC for patients with PC after NAT. We used the following terms: (pancreatic neoplasm OR pancreas cancer) AND (neoadjuvant therapy OR neoadjuvant Chemotherapy OR neoadjuvant chemoradiotherapy) AND (adjuvant chemotherapy OR adjuvant drug therapy). The detailed search strategy is summarized in Supplementary Table 1. In addition, all eligible studies were manually scrutinized. Two investigators independently evaluated the included studies. Any discrepancies in the literature search were settled by a consensual process.

Studies that meet the following criteria could be included: (1) the study design was a randomized controlled clinical trial or prospective/retrospective cohort study; (2) the study subjects were all patients with PC who undergo surgical resection after NAT (R2 resections < 2% per study; AC cycle > 3 months); (3) the studies had sufficient data to be analyzed including clinical characteristics and prognostic indexes such as OS, DFS, recurrence-free survival (RFS) and tumor-specific survival (CSS); (4) the study was published in English. Exclusion criteria are as follows: (1) meta-analysis, review, case report, comment, letter, conference abstract, and ongoing studies; (2) animal experiment and study not related to the subject matter of the article; and (3) studies that did not provide enough information to be included. For republished studies, only the most recent literature and relevant data were collected. Study design [Participants/Patients Intervention Control/Comparison Outcome Study design (PICOS)] components are detailed in Supplementary Table 2.

This study was conducted in accordance with the criteria established by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (supplementary PRISMA Checklist). It was registered in the PROSPERO (CRD42023461365) prospectively (16).

2.2 Data analysis

Two researchers (JHW and YKZ) independently extracted data and performed a literature quality assessment. Any disagreements were resolved by consensus through discussion with a third investigator. We extracted baseline characteristics from the included literature, including first author, study period, study country, year of publication, sample size, clinical characteristics, and clinical outcomes. The study selected OS and DFS/CSS/RFS as endpoints for this meta-analysis. The quality of the included studies was assessed using the Newcastle–Ottawa Scale (NOS) (17), where seven to nine points were rated as high-quality studies.

The hazard ratios (HRs) and 95% confidence intervals (95% CIs) were used to estimate the correlation between the administration of AC or not and the patient's prognosis in patients with Pancreatic Ductal Adenocarcinoma (PDAC) undergoing surgical resection after NAT. In most studies, data such as HRs and 95% CIs could be collected directly. However, we used Tierney's method to derive estimates from survival curves for all studies without relevant prognostic indicators (18). The heterogeneity among the included studies was assessed by using the chi-squared (χ^2) test (Cochran's *Q*) and inconsistency index (I^2) (19). P-values < 0.05 or I^2 > 50% indicated significant heterogeneity, in which case it was analyzed by using a random-effects model. Conversely, fixed-effects models were used when heterogeneity was small. P < 0.05 was considered statistically significant. Sensitivity analyses, funnel plots, and subgroup analyses were used to detect sources of heterogeneity. The subgroup analyzed factors included study style, the American Joint Committee on Cancer (AJCC) eighth N staging, tumor size (<2 cm and ≥2 cm), margin status, and histological grade. The results of the subgroup analysis are presented in Table 1. Begg's funnel plot test and Egger's test were used to test for publication bias in these studies (20). RevMan 5.3 (Cochrane Collaboration) and Stata 16.0 software (College Station) were used for this meta-analysis.

3 Results

The flowchart of the literature retrieval and screening process is presented in Figure 1. The systematic initial search yielded 3,063 relevant literatures, of which 955 were excluded due to duplication. Subsequently, 2,082 articles were elected by title and abstract, and 26 remaining articles were subjected to full text examination. Finally, a total of 15 articles that met the inclusion criteria were included in our meta-analysis, and a pooled analysis of 21,113 patients was conducted (7,794 patients in the AC group and 13,319 patients in the non-AC group). Three of these studies are retrospective ones using prospectively maintained databases (21–23), and the other 12 were retrospective cohort studies (14, 15, 24–33). It was worth noting that the study by Bolm et al. analyzed two sets of data separately. The included studies were published between 2017 and 2023 and conducted in five countries: 10 in the United States (14, 15, 21–24, 26, 28, 30, 31), 2 in China (32, 33), and 1 each in South Korea (27), Italy (29), and The Netherlands (25). Table 2 provides a summary of the main characteristics and NOS scores of the included studies. The median NOS score was 7 (ranging from 6 to 9) (34), and 10 studies were assessed as high-quality. Supplementary Table 3 presents the NOS assessment details for all included studies.

All 15 studies (14, 15, 21–33) with a total of 21,113 patients reported the influence of AC on OS. The pooled results of all the cohort studies using a random-effects model showed that AC was associated with significantly longer OS [HR = 0.80; 95% CI (0.75~0.86), P < 0.00001; Figure 2], accompanied by slight heterogeneity (I^2) = 48%, P = 0.02). Sensitivity analyses were performed to identify potential sources of heterogeneity, but no significant differences were found outside the limits of the 95% CI of the combined results. We also assessed the publication bias using funnel plots (Supplementary Figure S2C), Egger's test, and Begg's test and found no apparent publication bias for OS analysis (Egger's test: P = 0.104, Figure 3; Begg's test: P = 0.115, Supplementary Figure S2A).

Three studies (22, 27, 31), including 649 patients (332 patients in the AC group and 317 patients in the non-AC group), reported the DFS/CSS/RFS. The combined results obtained by the fixed-effects model showed a significant clinical benefit of AC on DFS/CSS/RFS in patients [HR = 0.53, 95% CI (0.41 ~ 0.69), P < 0.00001; Figure 3]. No heterogeneity was detected among the pooled results (I^2) = 0%, P = 0.95). Sensitivity analyses also did not find differences in the pooled results beyond the limits of 95% CI. Funnel plots (Supplementary Figure S2F), Egger's test, and Begg's test did not find any obvious publication bias between AC and DFS/CSS/RFS (Egger's test: P = 0.145, Supplementary Figure S2F; Begg's test: P = 1.00, Supplementary Figure S2D).

Subgroup analyses about oncological factors was performed to determine the benefiting subpopulation, which was helpful to making a rational decision in application of AC for patients underwent radical resection after NAT. Additionally, all of the following subgroup analyses were analyzed using a random-effects

TABLE 1 Subgroup meta-analysis of prognostic role of AC for OS in PC patients after NAT and radical pancreatectomy.

Factor	No. of study	No. of study		HR (95%CI)	P-value	Heterogeneity	
		AC	Non-AC			I^2 (%)	P-value
Study Style							
Retrospective	12	12	12	0.82	<0.00001	41%	0.2
Prospective	3	3	3	0.64	0.004	38%	<0.06
AJCC 8th N staging							
N0	9	9	9	0.79	<0.00001	0%	0.65
N1	6	6	6	0.78	<0.00001	32%	0.19
N2	4	4	4	0.69	0.11	75%	0.07
Tumor size							
<2cm	3	3	3	0.72	0.01	0%	0.48
<2cm	3	3	3	0.79	0.02	59%	0.09
Margin status							
R0	4	4	4	0.83	<0.00001	16%	0.31
R1	4	4	4	0.75	0.007	51%	0.11
Histological grade							
Low-grade group	3	3	3	0.97	0.87	0%	0.8
High-grade group	3	3	3	0.76	<0.00001	15%	<0.31

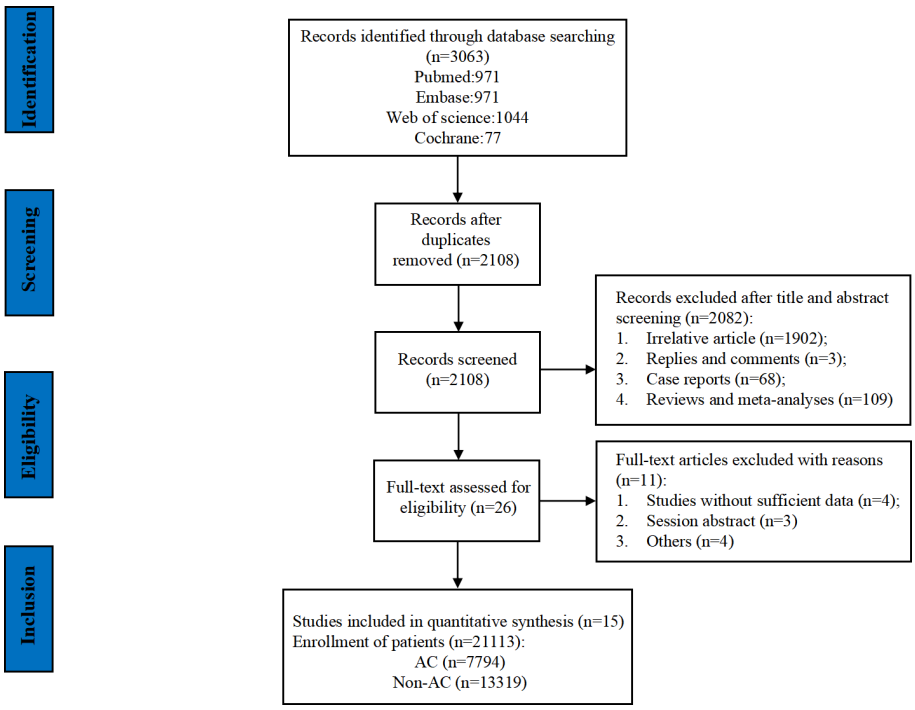

FIGURE 1
Flowchart of the systematic search and selection process.

TABLE 2 The characteristics of the included studies.

Study	Year	Country	Study style	Sample size	Patient		Years of enrolled patients	Median OS		Outcome	Follow-up times	NOS score
					Non-AC	AC		Non-AC	AC			
Olecki et al. (30)	2021	USA	Retrospective	3,897	2,405	1,492	2006–2015	23.22	26.04	OS	NA	7
Kamarajah et al. (26)	2021	USA	Retrospective	4,122	2,061	2,061	2004–2016	24.9	29.4	OS	NA	8
Ma et al. (28)	2019	USA	Retrospective	1,737	1,247	490	2004–2015	NA	NA	OS	Median time, 36 months	8
Sugawara et al. (14)	2023	CA	Retrospective	888	444	444	2010–2018	21.2	26.6	OS	Median time, 21.3 months	9
Bolm et al. (24)	2022	USA	Retrospective	202	100	102	2000–2018	17.8	21.3	OS	NA	6
Bolm* et al. (24)	2022	USA	Retrospective	4,749	4,172	577	2004–2018	26.4	35.4	OS	NA	6
Zhang et al. (33)	2022	USA	Retrospective	764	561	203	2011–2017	28.9	30.1	OS	Median time, 35.5 months	7
van Roessel et al. (15)	2020	EU	Retrospective	520	177	343	2012–2018	29	29	OS	Median time, 38 months	7
Hammad et al. (22)	2023	USA	Prospective	413	199	214	2010–2019	NA	NA	OS/PFS	NA	6
Barnes et al. (21)	2017	USA	Prospective	234	96	138	2009–2016	39	42.3	OS/PFS	Median time, 25.2 months	7
de Geus et al. (25)	2018	USA	Retrospective	1,357	833	524	2006–2013	27.1	27.5	OS	NA	6
Maggino et al. (29)	2023	ITA	Retrospective	373	123	250	2013–2017	35	36	OS	NA	6
Perri et al. (31)	2020	USA	Retrospective	122	61	61	2010–2017	17	42	OS/PFS	NA	8
Ivey et al. (23)	2022	USA	Prospective	427	186	241	2015–2019	20.4	28.7	OS/PFS	NA	6
Pu et al. (32)	2023	CA	Retrospective	1,194	597	597	2006–2019	25	30	OS/CSS	NA	8
Lee et al. (27)	2023	KR	Retrospective	114	57	57	2017–2020	23.5	31.5	OS/DFS	Median time, 26.6 months	9

NOS, the Newcastle-Ottawa Scale.
NA, Not Applicable.
*It was worth noting that Bolm et al. study analyzed two sets of data separately.

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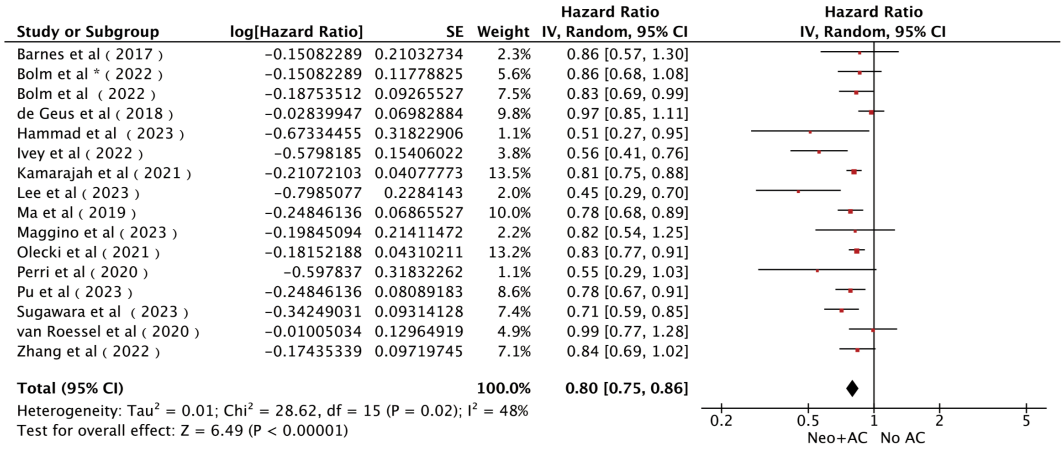

FIGURE 2
Forest plots of OS in patients with PC who received AC after NAT and radical pancreatectomy. *It was worth noting that Bolm et al. study analyzed two sets of data separately.

model (Table 2), and publication bias of the included studies was assessed using Funnel plots, Egger's test, and Begg's test (Supplementary Figure S3).

Firstly, we classified the included studies according to AJCC eighth N staging, and nine studies reported the effect of postoperative AC or OS in patients with a pathological N0 disease (14, 15, 21, 24, 26, 27, 29, 30, 32). The combined results using a random-effects model showed that patients with stage N0 could be significantly benefited from AC after NAT and surgery [HR = 0.79, 95% CI (0.74~0.84), P < 0.00001; Figure 4A]. Six studies reported the influence of postoperative AC on OS in patients with stage N1 (14, 24, 26, 29, 32, 33). The combined results also showed significant benefits of AC in these patients [HR = 0.78, 95% CI (0.72~0.85), P < 0.00001; Figure 4A]. Four studies reported the effect of postoperative AC in patients with stage N2 (26, 29, 32, 33). Surprisingly, AC did not prolong OS in patients with an N2 tumor after NAT and surgery [HR = 0.69, 95% CI (0.43~1.09), P = 0.11; Figure 4A].

Secondly, we analyzed the influence of tumor size on the effects of AC in patients underwent radical resection after NAT. Three studies were included (29, 30, 32), and the combined results indicated a significant improvement in OS associated with the use of AC regardless of the tumor size [<2-cm subgroup: HR = 0.72, 95% CI (0.55~0.94), P = 0.01; ≥2-cm subgroup: HR = 0.79, 95% CI (0.65~0.96), P = 0.02; Figure 4B].

Then, we analyzed the influence of the surgical margin status on the effects of AC in patients underwent radical resection after NAT. Four studies included (14, 26, 30, 33), and the combined results indicated that AC after NAT and resection increased OS compared with non-AC regardless of the margin status [R0 subgroup: HR = 0.83, 95% CI (0.77~0.88), P < 0.00001; R2 subgroup: HR = 0.75, 95% CI (0.61~0.92), P = 0.007; Figure 4C].

Finally, we analyzed the influence of tumor differentiation degree. Three studies were included, and the patients were classified to the low-grade group (well or moderate differentiation) and the high-grade group (poor or undifferentiation) according to the histological grade after completion of NAT. The combined results showed that the survival benefits of receiving additional AC after NAT were observed only in the high-grade group [HR = 0.76, 95% CI (0.66~0.87), P < 0.0001; Figure 4D] (14, 30, 32) but not in the low-grade group [HR = 0.97, 95% CI (0.66~1.42), P = 0.87; Figure 4D].

4 Discussion

To our knowledge, this study is the first one of systematic review and meta-analysis to assess the clinical implication of postoperative AC in patients with PC who underwent NAT and radical resection. The pooled analysis showed that AC was associated with a notably prolonged OS and DFS/CSS/RFS compared with non-AC. Subgroup analyses demonstrated that significant survival benefits of AC were observed regardless of the tumor size and resection margin status. However, the value of AC cannot be generalized in

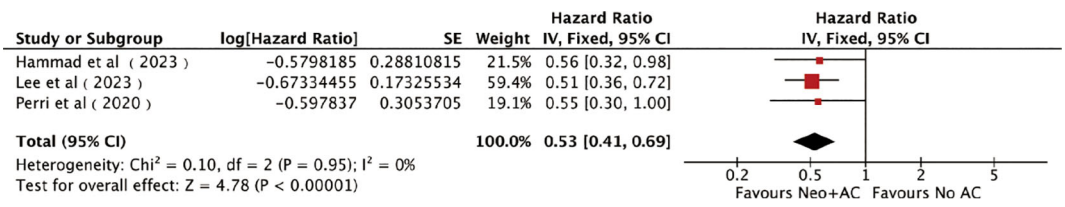

FIGURE 3
Forest plots of DFS/CSS/RFS in patients with PC who received AC after NAT and radical pancreatectomy.

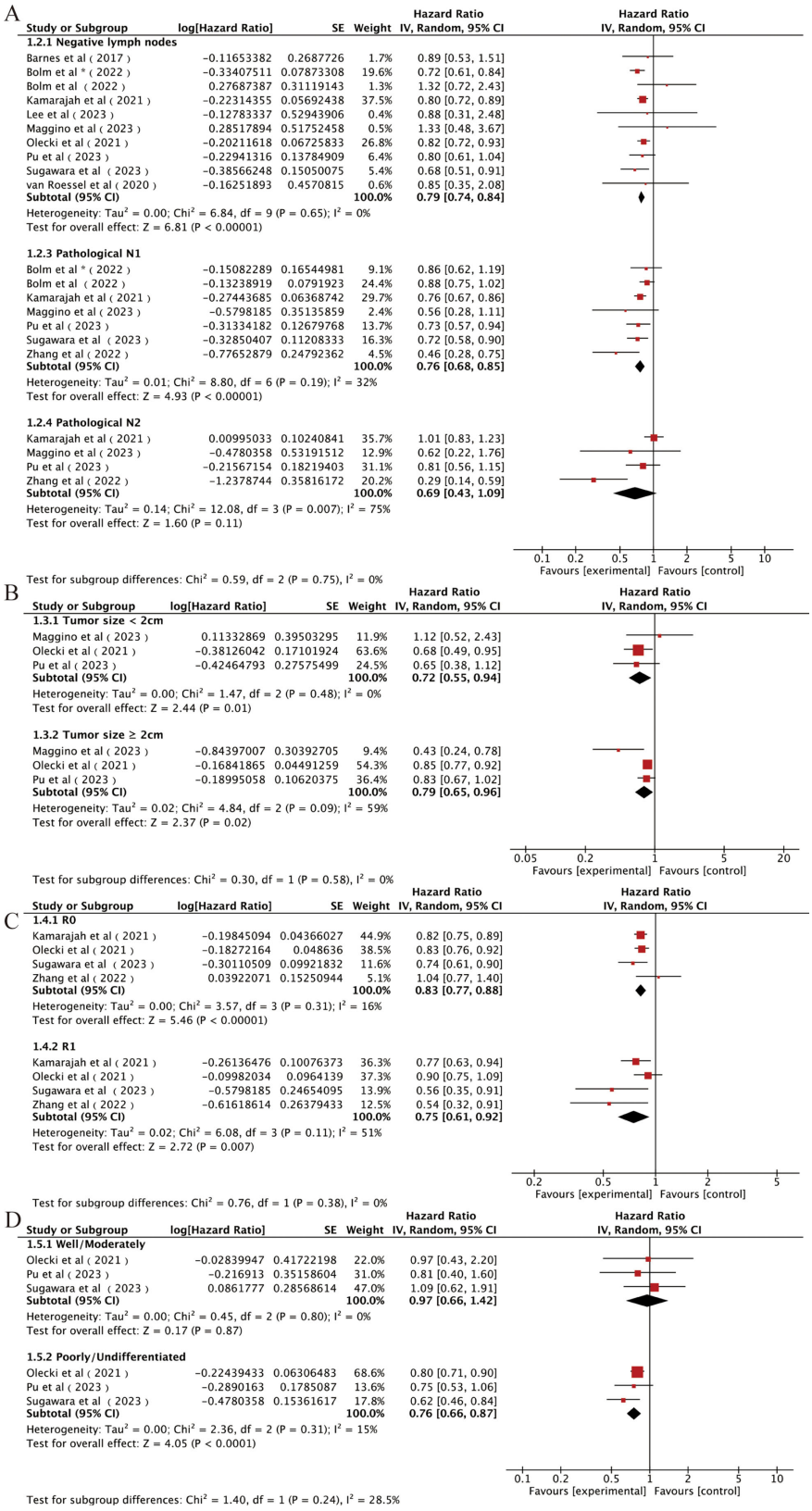

FIGURE 4
Forest plots of **(A)** N0, N1, and N2; **(B)** tumor size < 2 cm and tumor size > 2 cm; **(C)** R0 and R1; **(D)** well/moderate differentiation and poor/undifferentiation. *It was worth noting that Bolm et al. study analyzed two sets of data separately.

patients with different N staging and differentiation degree. We showed that AC benefited the patients with a stage N0, N1, or poorly/undifferentiated tumor in survival but not in patients with a stage N2 or well/moderately differentiated tumor. These findings were helpful to making a rational decision in selecting patients who underwent radical resection after NAT for further AC, which were also served as an important benchmark for future randomized controlled trials to well stratify patients.

PC has been emphasized as a systemic disease with a tendency to spread early (35, 36). Although surgery can offer a chance of cure for patients with (borderline) resectable PC, the presence of minimal residual and circulating tumor cells always results in early recurrence. It is worth noting that one of the most important roles of NAT, in addition to downstaging the primary tumor and increasing the rate of negative surgical margins, is the prevention of early postoperative metastasis in patients with PC (37). Furthermore, NAT has been shown to be efficient in enhancing systemic immunity and eradicating residual metastases in preclinical studies (38). Therefore, NAT can well overcome the limitations of the surgery-first approach, which has become a common practice in managing patients with borderline resectable PC and resectable tumors with high-risk factor and significantly prolongs the OS and Progression-free Survival (PFS) (39).

Noteworthily, there is evidence suggesting that the effects of trauma or immunosuppression in the host can cause metastasis of otherwise dormant tumor cells (40). As a trauma approach, preclinical evidence indicated that surgery could promote tumor metastatic mechanisms, potentially contributing to disease progression (41). AC could well remedy the shortcomings of surgery-only approach. However, whether the dormant tumor cells could be eliminated by NAT or the surgical-related trauma could arouse these cells was unknown. Whether AC could be replaced by NAT or AC could benefit the patients underwent radical resection after NAT was still unclear, especially considering the AC-related adverse effects and some postoperative patients unable to achieve the physical conditions required for AC, the value of AC in patients underwent radical resection after NAT was worth investigated.

Stereotypically, additional AC after NAT and surgery could provide an improvement in patients' prognosis. Actually, the indications for this paradigm remain controversial. Several retrospective cohort studies about this issue have been published in recent years. Barnes et al. (21) reported a retrospective study that indicated AC after NAT and surgery did not improve the OS of the whole[234]. Similarly, van Roessel et al. (15) showed no significant difference in survival between patients with PC received additional AC and those without AC. On the contrary, the study conducted by Sugawara et al. (14) who analyzed the data-based National Cancer Database (NCDB) and the another retrospective study performed by Kamarajah et al. (26) indicated that AC after NAT and surgery was significantly associated with an improvement in survival. After pooling the publications, we showed that AC in patients with PC who underwent NAT and radical resection improve the OS compared with non-AC.

Even so, the favorable outcomes of AC still cannot be generalized for all patients, which were influenced by the metastasis status of the regional lymph node and the differentiation degree. It is worth noting that the value of AC in patients with PC with different N stages is still under debate. Sugawara et al. (14) showed that AC had a better survival benefit in patients with any N staging. Other some studies indicated an improved survival of additional postoperative AC in patients with regional lymph node metastasis (15, 21). However, Pu et al. (32) found that only patients with PC with N1 disease could significantly benefit from additional AC after NAT and surgery, rather than patients with N0 or N2 disease. In addition, Zhang et al. (33) reported that additional AC was not only unrelated to the survival of patients underwent NAT and surgery but even shortened the OS in patients with positive nodal disease. The current pooled results demonstrated that AC benefited patients with a tumor in the stage N0 and N1 in survival but not in patients with a stage N2 tumor. Similarly, the value of AC in patients with different degrees of differentiation, which improved the OS in patients with a poorly/undifferentiated tumor but not in patients with a well/moderately differentiated tumor. It is hard to explain the heterogeneity of the outcomes of AC in patients with different N stages or degrees of differentiation, further clinical study should focus on this phenomenon. This meta-analysis has several limitations. First, all studies included in our analysis were cohort studies, and there was a lack of large randomized controlled trials to enhance the level of evidence. Second, most of the studies were analyzed using the NCDB and SEER databases, which limited our ability to obtain detailed information on the specific regimens and treatment cycles of NAT and AC, as well as tumor characteristics of patients with PC, such as resectability assessment, vascular invasion, and other recurrence-related factors. Additionally, due to the constraints of retrospective data, information regarding tumor recurrence and its impact following AC, beyond survival data, remains inaccessible. Consequently, further prospective trials are necessary to examine the benefits of AC after NAT and surgery more thoroughly. This limitation hindered our ability to conduct a comprehensive analysis of NAT and AC regimens and individualized therapy. Finally, aside from OS, there was inconsistent reporting of outcomes across the 15 studies included in the meta-analysis. Thus, we were unable to utilize all the included articles for our analyses of other indicators.

5 Conclusions

In conclusion, in this meta-analysis of 15 cohort studies, although AC showed survival benefit for patients with PC undergoing radical pancreatectomy after NAT, we still need to consider the lymph node stage and the degree of differentiation of the tumor when we gave AC to a patient. High-quality prospective randomized controlled studies are required to well disclose the value of AC in patients with PC undergoing radical pancreatectomy after NAT.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding authors.

Author contributions

JW: Conceptualization, Data curation, Methodology, Writing – original draft. YZ: Data curation, Investigation, Writing – review & editing. HW: Data curation, Writing – review & editing. WG: Formal analysis, Methodology, Writing – review & editing. CL: Formal analysis, Methodology, Writing – review & editing. YY: Writing – review & editing. HL: Writing – review & editing. FL: Conceptualization, Supervision, Writing – review & editing. LW: Funding acquisition, Writing – review & editing. JX: Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Writing – original draft.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2024.1429386/full#supplementary-material

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Case report: Combinations of immune checkpoint inhibitor, chemotherapy, and hyperthermia therapy avoid lymphatic recurrence in cholangiocarcinoma

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Cholangiocarcinoma is a malignancy known for its aggressiveness and limited treatment options. The malignant tumor behaviors include intrahepatic recurrence, regional lymph node (LN) metastasis, peritoneal carcinomatosis, and lung metastasis. Herein, we reported a case of lymphatic recurrence in an intrahepatic cholangiocarcinoma patient after surgery, adjuvant concurrent chemoradiotherapy (CCRT), who experienced a remarkable response to a combination therapy. However, the patient failed to undergo radiotherapy or other invasive local therapy and therefore received Opdivo (nivolumab) in combination with chemotherapy (FOLFOX) and modulated electro-hyperthermia. Notably, after these medical interventions, this patient had a complete response (CR) to treatments, in which no lymph node metastasis occurred, and a significantly decreased tumor marker, CA 19-9, level was found. This case highlights the potential of multiple anti-tumor therapies, including immune checkpoint inhibitors, chemotherapy, and hyperthermia, in managing challenging cholangiocarcinoma cases.

KEYWORDS

immune checkpoint inhibitor, hyperthermia, modulated electro-hyperthermia, cholangiocarcinoma, immunotherapy

Introduction

Cholangiocarcinoma is a formidable type of cancer that arises in the bile ducts and is known for its rarity and aggressiveness. However, patients with cholangiocarcinoma are often diagnosed in an advanced stage, which restricts treatment options and influences the overall survival time. Following curative resection, the median survival time is reported to

be 28–30 months, with a roughly 30% 5–year survival rate (1). The 5–year survival rate of intrahepatic cholangiocarcinoma with regional lymph node (LN) metastasis is about 9%, and only 3% for distant metastasis (2). One of the most significant challenges in managing this cancer is treating lymphatic recurrence following surgery and postoperative radiotherapy. Fortunately, a recent case report provides evidence that combining immunotherapy, chemotherapy, and modulated electro-hyperthermia may be an effective strategy for dealing with local lymphatic recurrence in cholangiocarcinoma (3).

Conventional treatments for cholangiocarcinoma, such as chemotherapy with gemcitabine and cisplatin, typically result in modest response rates and limited progression-free survival (PFS). For unresectable cholangiocarcinoma, the median overall survival (OS) with this regimen is less than one year, and the 5-year survival rate ranges from 20% to 35% depending on various factors like tumor location and stage (4). Response rates to systemic chemotherapy alone are generally low, with PFS often being less than six months. Studies have shown that adjuvant chemotherapy, despite its use, has not consistently demonstrated significant improvements in overall survival for all patients. The variability in outcomes is influenced by factors such as surgical margins, lymph node involvement, and the presence of metastatic disease (5). Locoregional therapies, including ablation, radioembolization, and chemoembolization, offer alternative strategies, particularly for patients who are not candidates for surgery. These therapies can provide local control and symptom relief, and in some cases, they achieve comparable outcomes to surgical resection for early-stage disease (6). The integration of newer treatment modalities, such as immunotherapy and targeted therapy, is being explored to improve response rates and survival outcomes in cholangiocarcinoma. However, the effectiveness of these treatments varies, and more research is needed to establish their role in standard care (4).

Case presentation

A 62-years-old male had history of hypertension under regular medication control. There were no other underlying medical comorbidities that this patient was suffering from diabetes, renal disease, cerebrovascular disease, hepatitis B/C carriers, congestive heart failure, or any other neoplastic diseases. His lifestyle factors included a regular, balanced diet, moderate stress level, no alcohol or tobacco use, no insomnia, and moderate physical activity. He initially presented with mild abdominal dull pain, and a computed tomography (CT) scan revealed a large intrahepatic tumor measuring 7–8 cm in size. It might have involved LNs in February 2021. At that time, tumor marker CA 19-9 exceeded 3000 U/mL (ranging from 0–37 U/mL in normal individuals). He underwent hepatectomy along with LN dissection in March 2021, followed by postoperative concurrent chemoradiotherapy (CCRT). The pathology report demonstrated a 7 cm cholangiocarcinoma with 1 in 9 regional LN metastasis, pT2N1, stage IIIb (AJCC 8th edition). Until July 2021, adjuvant CCRT was carried out using a 4500cGy/25fx radiation dose to the tumor bed, lymphatic region, and gemcitabine 800-1000 mg/m^2 for seven cycles. CA 19-9 reached a nadir of 27 U/mL then. However, less than three months after the initial treatment, the patient experienced a single regional LN recurrence, measuring a maximum of 2.76 cm. It was situated between the pancreas, duodenum, superior mesentery artery, inferior vena cava, and abdominal aorta. CA 19-9 rapidly increased to 195 U/mL in October 2021. Because of the prior radiation exposure, there was no room for further radiotherapy. It was also impractical to use any other invasive local therapies.

Treatment approach

Because of the patient's limited treatment options, a multidisciplinary team recommended a combination therapy to target the recurrent lymphatic metastasis. Immunotherapy with nivolumab, a PD-1 inhibitor, and chemotherapy with the FOLFOX regimen were chosen based on their known benefits in treating cholangiocarcinoma. Additionally, modulated electro-hyperthermia (mEHT) was employed to enhance the effectiveness of these treatments by selectively targeting cancer cells and making them more susceptible to therapy. The treatment protocol was described as follows (Figure 1).

Immune checkpoint inhibitor: The patient was initiated on nivolumab (Opdivo), a programmed cell death protein-1 (PD-1) immune checkpoint inhibitor. Nivolumab was administered 100 mg intravenously every three weeks for six cycles until February 2022. This immunotherapy aims to activate the patient's immune system to recognize and attack cancer cells.

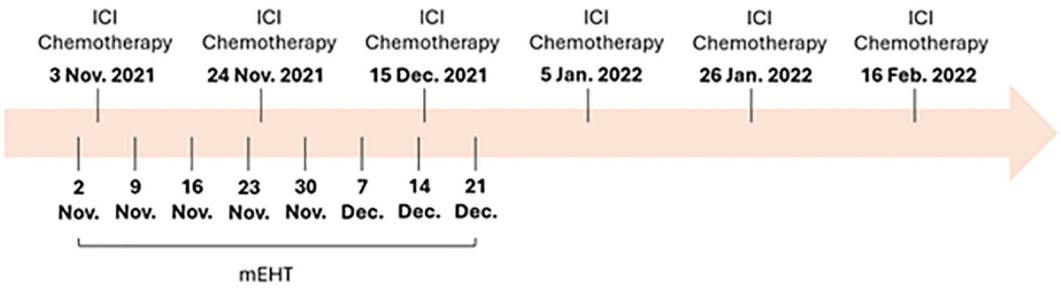

FIGURE 1
The treatment timeline of this case. ICI, Immune checkpoint inhibitor; mEHT, modulated electro-hyperthermia.

Chemotherapy: The patient underwent intravenously six cycles of chemotherapy (FOLFOX) every three weeks until February 2022, consisting of a platinum-based regimen tailored to cholangiocarcinoma. The FOLFOX regimen included oxaliplatin 85 mg/m^2, leucovorin 400 mg/m^2, and fluorouracil (5-FU) 2400 mg/m^2 bolus, followed by 5-FU 600 mg/m^2 as a 46-hour continuous infusion. Chemotherapy aimed to further debulk the tumor and sensitize it to immune checkpoint inhibition.

Modulated electro-hyperthermia (mEHT): The patient underwent a total of eight sessions of modulated electro-hyperthermia between November 2, 2021, and December 21, 2021, a hyperthermia treatment that applies controlled electromagnetic fields to target cancer cells selectively. The duration of each mEHT session lasted for 60 minutes. The EHY-2000 model of the Oncotherm machine was used for the mEHT treatments. The machine operated at a frequency of 13.56 MHz. The power output was maintained below 150 watts to ensure selective targeting of cancer cells while sparing healthy tissues. The mEHT treatments were administered specifically to the site of lymphatic recurrence. This approach leverages controlled electromagnetic fields to increase the temperature of cancer cells selectively, causing stress on their cell membranes and leading to cell death. This selective heating enhances the effectiveness of concurrent treatments, such as immunotherapy and chemotherapy, by making the cancer cells more susceptible to these therapies.

Outcome

The combined treatment approach yielded remarkable results, including a complete response to treatments and a decreased tumor marker.

Complete response: Imaging studies revealed complete regression of LN metastasis in response to the combined therapy regimen. The lymphatic recurrence was no longer detectable. Twenty-six months after recurrence, a CT scan in December 2023 still demonstrated no evidence of disease (Figure 2).

Tumor Marker Response: Tumor marker CA 19-9, which had initially elevated at 195 U/mL, exhibited a significant decrease, reaching 12.9 U/mL after completing the treatment regimen. In the

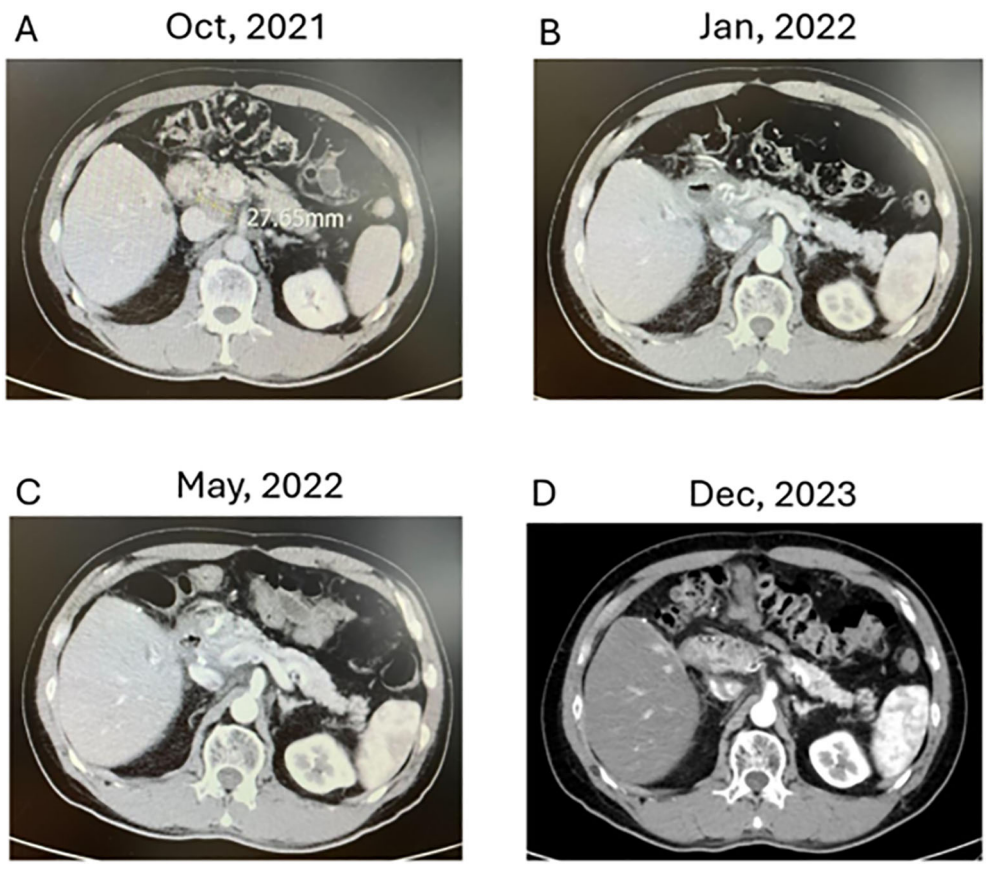

FIGURE 2
Evolution of Cholangiocarcinoma Through Multimodal Therapy **(A–D)** depict axial CT images demonstrating the response of lymphatic recurrence in a cholangiocarcinoma patient over time. (**A**: October 2021): Displays a single lymph node recurrence measuring approximately 2.76 cm amid critical anatomical structures post-adjuvant CCRT. (**B**: January 2022): Reveals a marked decrease in lymph node size, nearing complete response, following the initiation of combined modality treatment, including immune checkpoint inhibitors, chemotherapy, and modulated electro-hyperthermia. (**D**: December 2023): Illustrates sustained therapeutic success with no detectable signs of recurrence upon seven months of follow-up. (**D**: December 2023): At 26 months post-recurrence, comprehensive imaging confirms the absence of lymphatic or other metastases, supporting the patient's status in clinical remission.

long-term follow-up, CA 19-9 reached a nadir of 11.2 U/mL in December 2023. As of April 2024, there has been no recurrence with a stable CA 19-9 value of 10.6 U/mL (Figure 3), and the disease-free period lasted 29 months.

Discussion

Cholangiocarcinoma often presents a challenging clinical scenario when the cancer reoccurs, especially in the lymphatic system. In such cases, conventional treatments like radiation therapy and salvage surgery have limited efficacies, and alternative approaches should be taken into consideration This case study demonstrated how a multimodal approach that combines immune checkpoint inhibitors, chemotherapy, and mEHT therapy can achieve a favorable complete response and possibly cure the disease. The combined intervention approach targeted and activated the patient's immune system to fight against cancer cells while minimizing the side effects that usually accompany such treatments. This novel approach offers a promising new avenue for managing cholangiocarcinoma recurrence in the lymphatic system.

More and more evidence has revealed the existence of oligo recurrence or oligometastatic, which is a transitional state between limited primary cancer and polymetastatic cancer. In this state, local therapy for metastatic lesions leads to a satisfactory survival rate on par with non-metastatic disease (7). Traditionally, radiotherapy, ablation therapy, and surgery are deemed to salvage local therapies for oligo-recurrent tumors. Recently, local electro-hyperthermia has also been introduced clinically as an alternative approach for the focal treatment of tumors, especially when radiotherapy is not feasible. Electro-hyperthermia could serve both curative and palliative purposes. Combining electro-hyperthermia with systemic therapy typically results in a higher response rate than systemic therapy alone. The mechanisms underlying electro-hyperthermia involve excitement and disruption of the cancer cell membrane, increasing drug infiltration and anti-cancer sensitivity, and creating massive cancer apoptosis (8). For advanced cholangiocarcinoma, immunotherapy has shown its efficacy when combined with chemotherapy, and this combination treatment approach has emerged as the new standard (9). The effectiveness of the immune response and cytotoxicity would be maximized if electro-hyperthermia could be added to this combined approach, and it may be possible to achieve a higher rate of complete response, curative status, and more prolonged survival for recurrent cholangiocarcinoma.

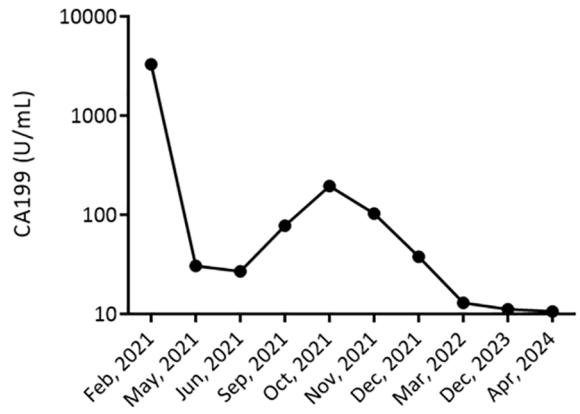

FIGURE 3
Longitudinal Monitoring of Tumor Marker CA 19-9 Levels. This figure displays the CA 19-9 tumor marker levels trend in a patient with cholangiocarcinoma from February 2021 to December 2023. The graph represents a significant decrease in CA 19-9 levels, from >3000 U/mL in February 2021, indicating a high tumor burden, to stabilizing at 13 U/mL by December 2023. The marked reduction in CA 19-9 levels post-treatment suggests an effective response to the therapeutic regimen.

The benefits of hyperthermia in cancer treatment, particularly in enhancing immune responses and delaying drug resistance, have been well-documented in various studies. Hyperthermia, mainly used in the "fever range" (41-43°C), can influence cell membrane fluidity and stability and trigger a heat shock response. This response induces heat shock proteins (HSPs), which can protect tumor cells against apoptosis triggered by oxidative stress (10). Interestingly, hyperthermia at this temperature range can also promote apoptosis in tumor cells, balancing pro- and anti-apoptosis processes. This mode of cell death, known as immunogenic cell death (ICD), can induce an inflammatory response and enhance the immune system's recognition of cancer cells (11). The process involves the release of damage-associated molecular patterns (DAMPs), such as high mobility group box 1 (HMGB1) and calreticulin (CRT), which are critical for the immunogenicity of cell death (12). The hyperthermia-induced elements are crucial because they are responsible for initiating tumor-specific immune responses, mainly by activating Toll-like receptor-4 (TLR-4) signaling pathways. HSPs, a group of highly conserved chaperone proteins, are among the most critical DAMPs induced by hyperthermia. These proteins, predominantly when overexpressed after hyperthermia, are associated with enhanced immune response (12). Furthermore, hyperthermia can suppress resistance pathways in cancer stem cells, making them more susceptible to other treatments like radiation therapy. This is particularly beneficial for bulky or refractory cancers. The cancer stem cell population plays a crucial role in disease recurrence due to its resistance to conventional therapies. By targeting these cells, hyperthermia can potentially improve treatment outcomes and reduce the likelihood of cancer recurrence (13).

Hyperthermia increases the expression of HSPs within tumor cells. HSPs play a crucial role in protein folding and protection under stress conditions, but they also enhance the immunogenicity of cancer cells (10, 14). When HSPs are overexpressed on the surface of tumor cells, they can improve antigen presentation, making the cancer cells more recognizable to the immune system. This heightened visibility enhances the effectiveness of immune checkpoint inhibitors like nivolumab, which relies on the immune system's ability to identify and attack cancer cell (12). Hyperthermia can cause the release of tumor antigens due to the stress and subsequent apoptosis or necrosis of cancer cells. The release of these antigens into the tumor microenvironment aids in the activation of dendritic cells and the subsequent priming of T-cells,

which are crucial for the immune response. This process is particularly beneficial when combined with immunotherapy, as the immune checkpoint inhibitors can further potentiate this immune activation by preventing the downregulation of immune responses (15). Hyperthermia increases the permeability of tumor vasculature, enhancing the delivery and penetration of chemotherapeutic agents into the tumor. This effect can be particularly significant in dense tumor tissues where drug delivery is often challenging. By improving the distribution of drugs like those in the FOLFOX regimen, hyperthermia ensures that a higher concentration of the chemotherapeutic agents reaches the tumor cells, thereby increasing their efficacy (16). While the positive outcomes in this case are encouraging, it is important to consider the generalizability of these results. Factors such as the patient's overall health, genetic profile, and tumor characteristics can influence treatment efficacy. In our case, the patient had a specific set of conditions and previous treatments that may not be representative of all cholangiocarcinoma patients. To generalize these findings, larger studies involving diverse patient populations are necessary. Future research should aim to identify biomarkers that predict response to mEHT combined with immunotherapy and chemotherapy, ensuring that the right patients can benefit from this treatment approach. The combination of mEHT with immunotherapy and chemotherapy offers a promising approach for treating advanced cholangiocarcinoma. By enhancing immune response, improving drug delivery, and increasing tumor antigen presentation, this multimodal therapy can provide substantial benefits. However, further research is needed to validate these findings in larger, more diverse patient populations and to optimize treatment protocols for different clinical scenarios. Integrating this approach into the current treatment landscape requires careful consideration of individual patient factors and ongoing advancements in cancer therapy.

This case report highlights the significant positive outcomes achieved through the combination of immune checkpoint inhibitor (nivolumab), chemotherapy (FOLFOX), and mEHT in treating lymphatic recurrence of cholangiocarcinoma. While these results are promising, it is important to acknowledge potential selection biases inherent in case reports and to compare our findings with other published cases and studies examining similar treatment combinations to provide a balanced perspective on treatment efficacy and patient variability. In this instance, the positive outcomes—complete regression of lymph node metastasis and significant reduction in tumor marker CA 19-9 levels—might not be representative of the broader patient population with cholangiocarcinoma. Factors such as the patient's overall health, underlying conditions, and specific tumor biology could have contributed to the favorable response observed in this case. Without a larger cohort for comparison, it is challenging to determine whether these results are widely applicable. To provide a more comprehensive view, it is essential to compare our findings with other studies that have explored similar treatment regimens. Studies such as the KEYNOTE-028 and KEYNOTE-158 trials have investigated the efficacy of pembrolizumab (an immune checkpoint inhibitor) in combination with chemotherapy for advanced biliary tract cancers, including cholangiocarcinoma. These studies have reported varying degrees of success, with some patients showing significant tumor reduction and prolonged PFS, while others did not respond as favorably (17). Sahai et al. reported on combining nivolumab with gemcitabine and cisplatin in advanced biliary tract cancers. The OS was 10.6 months, with a median PFS of 6.6 months, indicating that while some patients benefit from the combination, others may experience limited efficacy (18). Research on hyperthermia combined with radiotherapy and chemotherapy has shown enhanced treatment responses in various cancers. For example, Issels et al. demonstrated that adding regional hyperthermia to chemotherapy improved survival outcomes in patients with high-risk soft tissue sarcoma. This suggests that hyperthermia can potentiate the effects of systemic therapies, similar to the outcomes observed in our case (19). While our case report demonstrates the potential benefits of combining mEHT with immune checkpoint inhibitors and chemotherapy, it is crucial to recognize that these findings may not be universally applicable. The variability in patient responses highlights the need for personalized treatment approaches and the importance of conducting larger, randomized clinical trials to validate these results.

The management of cholangiocarcinoma, particularly in advanced stages, remains a formidable challenge due to its aggressive nature and limited treatment options. Recent advancements in cancer therapy have highlighted the potential benefits of combining various modalities to enhance treatment efficacy and improve patient outcomes. Our case report contributes to this evolving landscape by presenting a successful instance of combining immune checkpoint inhibitors, chemotherapy, and mEHT to treat lymphatic recurrence in cholangiocarcinoma. The positive outcomes observed in our case report contribute to the growing body of evidence supporting the use of multimodal therapies in cancer treatment. While our findings are based on a single case, they provide a valuable proof-of-concept that can inform future research and clinical practice. The success of the combination therapy in achieving complete regression of lymph node metastasis and significant tumor marker reduction highlights the need for further investigation through larger clinical trials.

Molecular testing, such as genomic profiling, can identify specific mutations and biomarkers that might inform targeted therapy options. These include mutations in genes like IDH1/2, FGFR2 fusions, and others with potential targeted treatments. However, in this study, we failed to provide genomic data, such as NGS results reporting the patient's mutational profile and the implication of mutations on responses. We speculated that the availability and integration of molecular testing into routine clinical practice can vary significantly based on regional healthcare infrastructure and resources. In many regions, the cost and accessibility of comprehensive molecular profiling are limiting factors. Despite the potential benefits of targeted therapies identified through molecular testing, the multidisciplinary team, in this case, prioritized the use of accessible treatments with established efficacy.

The patient was thoroughly informed about the potential risks and benefits associated with the combined treatment regimen of immune checkpoint inhibitors, chemotherapy, and mEHT. This discussion was designed to provide a balanced view of what the

patient could expect. The healthcare team explained that the combined treatment could potentially enhance therapeutic effectiveness due to the synergistic effects of mEHT with chemotherapy and immunotherapy. The patient was informed about the possibility of significant tumor shrinkage and the potential for complete regression of lymph node metastasis, as well as the overall improvement in survival and quality of life based on current research and similar case reports. The patient was also made aware of the possible side effects of chemotherapy and immunotherapy, including fatigue, nausea, and immune-related adverse events. Additionally, the risks associated with mEHT, such as skin reactions and discomfort during the procedure, were discussed. The patient was informed about the uncertainty regarding long-term outcomes and the overall effectiveness of this novel combination therapy. By involving the patient in the decision-making process and ensuring a thorough understanding of the treatment's potential risks and benefits, the ethical transparency of this case report is enhanced. Respecting the patient's autonomy throughout the process allowed them to make an informed decision about their treatment.

Our case report highlights the potential benefits of combining immune checkpoint inhibitors, chemotherapy, and mEHT in the treatment of advanced cholangiocarcinoma. To build on these findings and advance the field, we propose several directions for future research. The primary objective of future research should be to rigorously evaluate the efficacy and safety of the combined treatment regimen in a larger patient cohort. This can be achieved through multi-center, randomized controlled trials comparing the combination of mEHT, immunotherapy (such as nivolumab), and chemotherapy (such as FOLFOX) with standard treatments. Primary endpoints for these trials could include PFS, OS, and objective response rate (ORR), while secondary endpoints might assess quality of life, treatment tolerability, and biomarkers for treatment response. To validate the results observed in our case report across a more diverse and extensive patient population, prospective cohort studies involving patients with advanced cholangiocarcinoma receiving the combination therapy should be conducted. These studies should collect detailed data on patient demographics, treatment regimens, outcomes, and adverse events. Comparative effectiveness analysis can then be performed to evaluate the real-world impact of the combined treatment on survival and disease progression, with subgroup analyses identifying patient characteristics associated with better outcomes. Preclinical studies are essential to understand the underlying mechanisms by which mEHT enhances the effects of immunotherapy and chemotherapy. Laboratory studies using cell lines and animal models of cholangiocarcinoma should investigate the biological processes involved. Key areas of investigation should include the role of heat shock proteins in tumor immunogenicity and response to immune checkpoint inhibitors, the impact of hyperthermia on tumor vasculature and drug delivery efficiency, and the interactions between mEHT-induced stress responses and immune activation. Identifying predictive biomarkers that can help select patients most likely to benefit from the combined treatment regimen is crucial. Correlative studies within clinical trials and observational studies should collect tissue and blood samples for molecular analysis. Using genomics, proteomics, and immunohistochemistry, researchers can identify biomarkers associated with response to mEHT, immunotherapy, and chemotherapy. Potential biomarkers could include specific genetic mutations, protein expression levels, and immune cell infiltration patterns. Future research should focus on validating and expanding upon the promising results observed in this case report. By conducting rigorous clinical trials, larger observational studies, and detailed preclinical investigations, we can better understand the interactions between these therapies and optimize treatment protocols. Identifying predictive biomarkers will further enable personalized treatment approaches, ultimately improving outcomes for patients with advanced cholangiocarcinoma. These efforts will contribute significantly to the advancement of cholangiocarcinoma treatment and potentially benefit other cancer types as well.

Conclusion

The effective treatment of lymphatic recurrence in cholangiocarcinoma by combining immunotherapy, chemotherapy, and modulated electro-hyperthermia emphasizes the significance of innovative and multidisciplinary approaches in complex cancer cases. More research and clinical studies are needed to explore the potential of such multiple strategies in enhancing outcomes for patients with advanced cholangiocarcinoma.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Ethics statement

Ethical approval was not required for the studies involving humans because the patient provided written informed consent to participate in this study. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

H-JC: Conceptualization, Data curation, Formal analysis, Writing – original draft, Writing – review & editing. C-HK: Investigation, Methodology, Project administration, Resources, Writing – review &

editing. Y-SW: Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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Conflict of interest

Y-SW was employed by Uni-Pharma Co-Ltd.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Sorafenib combined with tarexib for first-line treatment of unresectable hepatocellular carcinoma and its predictive role and correlation with PD-L1 CTCs

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Background: This study aims to evaluate the safety efficacy of combining the PD-1 antibody Tirelizumab with Sorafenib in the treatment of advanced hepatocellular carcinoma. Additionally we are committed to investigating the relationship between circulating tumor cell (CTC) counts/PD-L1 expression the prognosis of patients with advanced hepatocellular carcinoma.

Methods: This study included 32 patients with unresectable primary liver cancer who received treatment with Tislelizumab in combination with Sorafenib. Tislelizumab was administered via intravenous injection at a dose of 200 mg every 3 weeks, while Sorafenib was given orally at a dose of 400 mg twice daily. Patients were evaluated every 3 cycles (9 weeks) to assess the safety and efficacy of the treatment regimen. Prior to enrollment, all patients underwent CTC counting and assessment of PD-L1 expression in circulating tumor cells. The primary endpoint was the objective response rate (ORR), evaluated by the investigator according to the RECIST v1.1 criteria. Secondary endpoints aimed to assess the relationship between circulating tumor cell (CTC) counts or programmed death ligand 1 (PD-L1) expression and the prognosis of patients with advanced hepatocellular carcinoma.

Results: As of November 2022, a total of 32 patients have been enrolled in the study and received combination treatment. Among the 32 patients, 31 (96.8%) tested positive for circulating tumor cells (CTCs), with counts ranging from 1 to 45 and a median of 7 (3, 11). PD-L1-positive CTCs were detected in 25 patients (78.1%). All 32 patients were followed up for 2 to 14 months, with a median follow-up time of 6 months. Correlation analysis revealed that distant metastasis, vascular invasion, and the presence of more than 5 CTCs were significantly associated with PD-L1-positive CTCs. The one-year overall survival rates for patients with PD-L1-positive CTCs and those with PD-L1-negative CTCs were 78.5% vs 64.3% (P = 0.309). Additionally, the one-year overall survival rates for the group with rising CTC counts compared to the group with stable or declining counts were 34.3% vs 90% (P = 0.063).

Conclusion: The combination of Tislelizumab and Sorafenib demonstrates promising antitumor activity in the first-line treatment of hepatocellular carcinoma, with a relatively high objective response rate (ORR) and acceptable

safety profile. Baseline CTC PD-L1 positivity can serve as a predictive marker for selecting hepatocellular carcinoma patients for PD-1/PD-L1 blockade therapy, and dynamic measurement of CTC changes can be used to monitor treatment efficacy.

KEYWORDS

hepatocellular carcinoma, Tirelizumab, Sorafenib, circulating tumor cells, PD-L1

1 Introduction

Primary liver cancer is one of the most common malignant tumors worldwide, accounting for 6% of all new cancer cases each year. It ranks fifth in incidence and third in cancer-related mortality globally (1). China is a high-incidence area for liver cancer, with its incidence and mortality rates ranking fourth and third, respectively, among malignant tumors, posing a significant threat to the health and safety of the Chinese population (2). For patients with unresectable liver cancer or those with metastasis, treatment options include immunotherapy and targeted therapy. Tirelizumab is a PD-1 monoclonal antibody and belongs to the class of tumor immunotherapeutics known as immune checkpoint inhibitors. It is capable of inhibiting tumor growth, development, invasion, and metastasis (3). Sorafenib tosylate exerts a dual antitumor effect by directly inhibiting tumor cell proliferation through the blockade of the RAF/MEK/ERK-mediated signaling pathway (4). It also indirectly suppresses tumor cell growth by inhibiting the formation of new blood vessels through the blockade of VEGFR and platelet-derived growth factor (PDGF) receptors. The combination of immune checkpoint inhibitors and VEGFR-targeted inhibitors has shown promising results, but research on the use of Tirelizumab in conjunction with Sorafenib is still limited. Furthermore, there are few factors available that can serve as predictive markers for prognosis in liver cancer treatment, making the study of PD-L1 positive circulating tumor cells (CTCs) particularly important. Therefore, this study focuses on patients with unresectable advanced hepatocellular carcinoma to explore the clinical efficacy, adverse reactions, and survival outcomes of Tirelizumab combined with Sorafenib. Additionally, we monitored the expression of PD-L1+ CTCs during treatment to investigate their potential as predictive biomarkers for HCC. The findings are reported as follows.

2 Data and methods

2.1 General information

Clinical data from 32 newly diagnosed patients with advanced liver cancer who visited the Department of Hepatobiliary Surgery at Shenzhen Hospital of the Chinese Academy of Medical Sciences from February 2022 to February 2023 were analyzed. This study was approved by the hospital's Medical Ethics Committee, and informed consent was obtained from both the patients and their families.

2.2 Inclusion criteria:

(1) Diagnosis confirmed based on the relevant diagnostic criteria outlined in the "Guidelines for the Diagnosis and Treatment of Primary Liver Cancer (2019 Edition)" through pathological, cytological, and imaging examinations (5). (2) At least one measurable lesion based on the Response Evaluation Criteria in Solid Tumors (RECIST 1.1). (3) Barcelona Clinic Liver Cancer (BCLC) staging: Stage B or C. (4) No allergy to the study medications. (5) Expected survival time > 3 months. (6) Complete medical records.

2.3 Exclusion criteria:

(1) Severe disease of essential organs such as the heart, brain, liver, or kidneys. (2) Concurrent hematological, immunological, or neurological disorders. (3) Presence of other malignancies. (4) Existence of two or more liver diseases. (5) Currently undergoing other treatments. (6) Pregnant or breastfeeding women.

2.4 Methods

2.4.1 Treatment methods

Sorafenib tosylate (Manufacturer: Bayer AG) was administered orally at a dose of 0.4 g twice daily (0.25 g per tablet). Tislelizumab (Manufacturers: Boehringer Ingelheim (China) Co., Ltd. and Guangzhou Baiyunshan Pharmaceutical Holdings Co., Ltd.; Approval No.: National Drug Standard S20190045) was administered by intravenous infusion at a dose of 200 mg per session every 21 days, with a total volume of 10 mL (100 mg). Treatment continued until disease progression or intolerable adverse reactions occurred, with follow-up until December 2023.

In cases of severe treatment-related adverse events (TRAE) that rendered the patient unable to tolerate the medication, the drug dosage could be reduced or discontinued as necessary. Treatment could be resumed once the severity of the adverse reactions decreased or resolved.

2.4.2 CTC enrichment and PD-L1+ CTC detection

Circulating tumor cells (CTCs) were detected using the CTC 100 microfluidic chip platform (Jingzhen Biomedical (Shenzhen) Co., Ltd.), based on the principle of inertial focusing (6). During the first three days of drug treatment, 4 mL of peripheral blood was collected from enrolled patients using ACD blood collection tubes compatible with the CTC 100 platform, and the samples were processed within 6 hours at room temperature. First, plasma was removed by centrifugation, preserving the cellular layer, after which density gradient centrifugation was performed on the blood cells to obtain peripheral blood mononuclear cells (PBMCs). These PBMCs mainly consist of tumor cells and leukocytes. Subsequently, the CTC100 cell sorting system was used to enrich and isolate suspected CTCs (which may contain some residual leukocytes) from the PBMCs. All enriched suspected CTCs were fixed in 4% paraformaldehyde and subjected to immunofluorescence staining. The staining antibodies included DAPI, CD45, EpCAM, and PD-L1. After antibody staining, the cells underwent nuclear staining and were analyzed under a microscope. Cells identified as DAPI +/EpCAM+/CD45- that met certain morphological and size criteria were classified as CTCs, while those identified as DAPI+/EpCAM +/CD45-/PD-L1+ were classified as PD-L1 positive CTCs.

2.4.3 Observation indicators and evaluation criteria

Treatment efficacy was assessed according to the Response Evaluation Criteria in Solid Tumors (RECIST 1.1) (7):

Complete Response (CR): Disappearance of both target and non-target lesions for more than 4 weeks.

Partial Response (PR): A decrease of at least 30% in the sum of the longest diameters of target lesions compared to baseline, maintained for more than 4 weeks.

Progressive Disease (PD): An increase of more than 20% in the sum of the longest diameters of target lesions, along with the appearance of new lesions.

Stable Disease (SD): A situation where target lesions have not decreased enough to qualify as a partial response or have not increased enough to qualify as progressive disease.

The Objective Response Rate (ORR) was calculated as (number of complete responses + number of partial responses)/total number of patients × 100%. The Disease Control Rate (DCR) was calculated as (1 - number of progressions/total number of patients) × 100%.

Additionally, the study observed the occurrence of adverse reactions, including nausea and vomiting, bone marrow suppression, hepatic and renal toxic reactions, rash, hepatitis, pneumonia, diarrhea, hand-foot syndrome, and proteinuria. Furthermore, the median survival time and overall survival time were evaluated.

3 Results

3.1 General clinical characteristics

A total of 32 patients with hepatocellular carcinoma (HCC) who received Tirelizumab in combination with Sorafenib underwent PD-L1 CTC detection prior to treatment. Among them, the PD-L1+ CTC group consisted of 24 patients, all of whom were male. In this group, 23 patients were HBsAg positive, 23 had positive liver cirrhosis, and 22 had pre-treatment levels ≥ 400μg/L. The Child-Pugh scores were classified as Grade A in 7 patients, Grade B in 15 patients, and Grade C in 3 patients. Additionally, 15 patients had tumors with a diameter > 5 cm, and 15 patients had ≥ 3 tumors. There were 20 cases of distant metastasis and vascular invasion in 19 patients, with the number of detected CTCs ≥ 5 per 10 mL in 18 cases. The PD-L1- CTC group included 7 patients. Correlation analysis revealed that distant metastasis, vascular invasion, and CTC count greater than 5 were significantly associated with PD-L1+ CTCs. Detailed baseline characteristics of the patients are shown in Table 1.

TABLE 1 The relationship between PD-L1 CTC and clinical pathological features.

Baseline characteristics	n	PD-L1 CTC		*P* value
		- (n=7)	+ (n=25)	
Age (years)				0.669
< 50y	14	4	10	
≥ 50y	18	3	15	
Sex				
Male	29	5	24	0.113
Female	3	2	1	
HBsAg				0.201
Negative	4	2	2	
Positive	28	5	23	
Liver cirrhosis				1.000
No	2	0	2	
Yes	30	7	23	
AFP before treatment				0.101
< 400μg/L	6	3	3	
≥ 400μg/L	26	4	22	
Child-Pugh				0.707
A	10	3	7	
B	18	3	15	
C	4	1	3	
Tumor size				0.669

(Continued)

TABLE 1 Continued

Baseline characteristics	n	PD-L1 CTC		*P* value
		- (n=7)	+ (n=25)	
Sex				
≤ 5cm	14	4	10	
>5cm	18	3	15	
Number of tumors				0.683
<3	12	2	10	
≥ 3	20	5	15	
Distant transfer				0.002*
Negative	10	5	5	
Positive	22	2	20	
Vascular invasion				0.032*
No	11	5	6	
Yes	21	2	19	
Number of CTCs (n=31)				0.022*
<5	12	5	7	
≥5	19	1	18	

*P<0.05. AFP, Alpha-fetoprotein. CTC, Circulating tumor cells.

3.2 Treatment safety and efficacy

As of the follow-up date, among the 32 patients receiving the combination therapy, the incidence of adverse reactions was 28.13% (9/32). The reported adverse reactions included 2 cases of rash, 2 cases of nausea and vomiting, 2 cases of bone marrow suppression, 1 case of hepatic and renal toxicity, 1 case of diarrhea, and 1 case of thyroid dysfunction (See Table 2 for details). The overall Disease Control Rate (DCR) was 46.88%. The DCR for patients in the PD-L1+ CTC group was 60.0% compared to 0% in the PD-L1- CTC group (P = 0.008), indicating a statistically significant difference. Additionally, the DCR for the CTC increase group was 37.5% compared to 60.0% in the non-increase group (P = 0.023), with no significant statistical difference observed. See Table 3 for details.

3.3 Survival status

All 32 patients were followed up, with follow-up durations ranging from 2 to 14 months and a median follow-up time of 6 months. The overall survival rates at 6 months and 1 year were 87.7% and 72.9%, respectively. The tumor-free survival rate at 6 months was 75.6%, while at 1 year, it was 5.8% (Figure 1). The one-year overall survival rates for patients in the PD-L1+ CTC group and the PD-L1- CTC group were 78.5% and 64.3%, respectively (P = 0.309), showing no significant statistical difference. In contrast, the one-year overall survival rates for the CTC increase group and the non-increase group were 34.3% and 90%, respectively (P = 0.063), indicating a statistically significant difference. See Figure 2 for details.

4 Discussion

Primary liver cancer is a highly prevalent malignant tumor in the digestive system, characterized by treatment difficulty, high mortality rates, and poor prognosis. Due to the insidious nature of liver cancer, most patients are diagnosed at an advanced stage, making surgical intervention challenging (8). Therefore, actively exploring treatment options for unresectable liver cancer is of significant importance for improving patient prognosis. Currently, tumor immunotherapy has received increasing attention and recognition as a treatment modality for advanced liver cancer. However, this therapy often has a prolonged response time, and a substantial portion of the patient population may either not respond to the treatment or experience delayed responses. As a result, a considerable number of patients may abandon treatment due to early disease progression (9). Although targeted therapies can elicit responses early in treatment, these responses tend to be of short duration and are often accompanied by the development of resistance (10). Considering the distinct characteristics of these two therapeutic approaches, the combination of tumor immunotherapy and targeted therapy shows promising applicability, as it can enhance response rates while providing sustained clinical benefits. This study explored the efficacy of the immune checkpoint inhibitor Toripalimab in combination with the targeted inhibitor Sorafenib for the treatment of patients with advanced liver cancer. After

TABLE 2 Adverse reactions and grading.

Adverse Effects	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
Rash	1	1	0	0	0
Nausea	2	0	0	0	0
Leukopenia	0	1	0	0	0
Thrombocytopenia	0	1	0	0	0
ALT elevation	1	0	0	0	0
Diarrhea	1	0	0	0	0
Hypothyroidism	1	0	0	0	0

TABLE 3 Efficacy of treatment based on PD-L1 CTC subgroups and changes in CTC counts.

Groups	n	Efficacy of Treatmen		*P* value
		DCR	PD	
PD-L1 CTC				0.008
Negative	7	0	7	
Positive	25	15	10	
Changes in CTC Counts				
no-Ascend	15	9	6	0.289
Ascend	16	6	10	

combination therapy, the overall survival rate for patients at 6 months was 87.7% and at 1 year was 72.9%. The tumor-free survival rate was 75.6% at 6 months and 5.8% at 1 year. These results are consistent with those reported in the literature, and the 6-month tumor-free survival rate even surpasses some of the current data, demonstrating good efficacy and safety.

CTCs are considered a source of tumor metastasis and recurrence. The prognostic value of CTCs has been established in breast cancer (11), prostate cancer (12), colorectal cancer (13), as well as small cell (14) and non-small cell lung cancer (15). Compared to existing imaging techniques and invasive procedures such as biopsies, CTCs offer advantages such as real-time monitoring, non-invasiveness, high sensitivity, and high specificity. Additionally, studies have shown that CTCs are an independent risk factor for hepatocellular carcinoma (HCC) and are significantly associated with patient prognosis. The assessment of CTC PD-L1 expression has broad potential applications for dynamically monitoring disease changes and evaluating treatment responses during immunotherapy. These conclusions are consistent with our research findings, which show that patients in the baseline CTC PD-L1(+) group have statistically superior overall survival and progression-free survival compared to those in the PD-L1(-) group (16, 17). In line with this, it has been reported that cancer patients with PD-L1-expressing CTCs in urothelial carcinoma (18) and melanoma (19) exhibit better treatment outcomes during immune checkpoint inhibitor (ICI) therapy. Conversely, there are studies reporting that meta-analyses indicate poorer prognoses for cancer patients with PD-L1-expressing CTCs (20–22). The discrepancies in these reports may be attributed to differences in cancer types, methods of CTC collection, and assessment techniques for PD-L1 expression, necessitating further validation through additional multi-center studies with larger sample sizes.

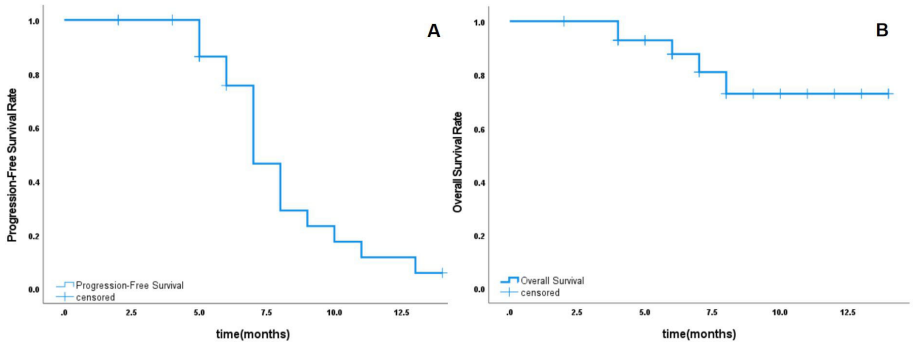

FIGURE 1
Overall survival rate and tumor-free survival rate. **(A)** The tumor-free survival rate at 6 months and 1 year. **(B)** overall survival rates at 6 months and 1 year.

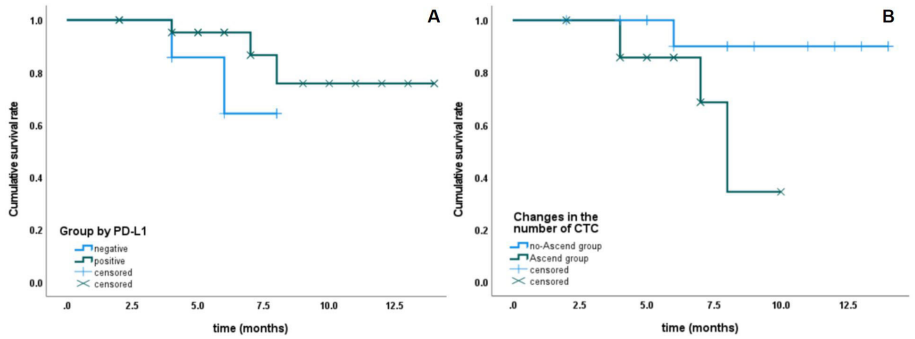

FIGURE 2
The relationship between PD-L1 expression and changes in CTC number with survival rate. **(A)** Patients with PD-L1-positive CTCs have bettersurvival rates than those with PD-L1-negative CTCs, although the difference is not statistically significant. **(B)** Patients with stable or decreasing CTC counts have better survival rates than those with increasing CTC counts, although P= 0.063).

This study is an exploratory investigation into the combination of targeted therapy aimed at the VEGFR and RAF/MEK/ERK signaling pathways with PD-1 monoclonal antibody treatment for advanced liver cancer. Additionally, it evaluates whether monitoring the expression of PD-L1 in CTCs can serve as a prognostic indicator for combined treatments in liver cancer. The aim is to provide guidance and insights for urgently needed novel combination therapies and monitoring indicators in clinical practice, laying a foundation for subsequent research, which holds significant scientific and clinical value. This plays a crucial role in formulating personalized treatment strategies for patients with advanced liver cancer, aiding in treatment decisions and monitoring disease progression. For patients with PD-L1+ CTC, combination therapy with immunotherapy and targeted therapy should be considered. If a patient's PD-L1+ CTC levels rise during treatment, it may suggest disease progression or treatment failure, signaling the need for timely adjustments in the treatment plan to improve patient outcomes. In the future, with the further development of liver cancer risk factors such as HCV, there will be an increasing need for precision treatment. For example, the combination of CTCs and different HCV genotypes may provide therapeutic information for personalized therapies (23), while insights from CTC PD-L1 expression can guide targeted treatment. Prevention strategies should emphasize screening and lifestyle modifications. By integrating these aspects, we can enrich the understanding of advanced liver cancer management, improving treatment outcomes and preventive measures from population health impact to molecular precision medicine.

From the findings of this study, it is evident that, from a safety perspective, the probability of adverse events (AEs) occurring during combination therapy did not increase. In terms of efficacy, the combination treatment of PD-1 inhibitors with targeted drugs demonstrated better clinical outcomes for patients with advanced primary liver cancer compared to monotherapy with PD-1 inhibitors. Additionally, PD-L1+ CTCs were associated with better disease control rates and survival rates in the context of combination therapy. However, it is important to emphasize that this study included only 32 samples and was based on single-center retrospective data, which may limit the representativeness of the findings. This may not fully reflect patient responses across different populations and clinical settings, and especially in subgroup analyses, some bias may occur. Moreover, subsequent treatments after tumor progression and factors such as patient comorbidities may affect the median survival time and overall survival outcomes, which could also impact the accuracy of the results. Therefore, future research with larger sample sizes, multi-center designs, and randomized controlled trials is needed to further validate our findings and explore the treatment effects and safety in different patient populations. Additionally, longer follow-up periods should be considered to more comprehensively assess the durability of the treatment and its potential long-term effects.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by Ethics Committee of Shenzhen Cancer Hospital, Chinese Academy of Medical Sciences. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

LX: Writing – original draft, Writing – review & editing. XC: Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Prognostic role of systemic inflammation response index (SIRI) in patients with pancreatic cancer: a meta-analysis

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Background: The significance of the systemic inflammation response index (SIRI) in predicting the prognosis of patients with pancreatic cancer (PC) has been extensively explored; however, findings remain controversial. As such, this meta-analysis was performed to more precisely determine the utility of the SIRI in predicting PC prognosis.

Methods: A comprehensive literature search of the PubMed, Web of Science, Embase, and Cochrane Library databases for relevant studies, published up to June 25, 2024, was performed. The primary and secondary endpoints were overall survival (OS) and progression-free survival (PFS), respectively. The prognostic utility of the SIRI in predicting PC prognosis was estimated by calculating pooled hazard ratios (HRs) and corresponding 95% confidence intervals (CIs).

Results: Seven studies comprising 1160 patients were included in the present meta-analysis. Pooled findings revealed that elevated SIRI was as a prominent prognostic marker of OS (HR 2.40 [95% CI 1.88–3.05]; p<0.001) and PFS (HR 1.95 [95% CI 1.19–3.21]; p=0.008) in patients diagnosed with PC. According to subgroup analysis, the SIRI remained an outstanding prognostic marker for OS, irrespective of region, sample size, study center, study design, TNM stage, cancer type, cut-off value, treatment, or survival analysis type (all p<0.05). Moreover, based on subgroup analysis, the SIRI demonstrated significant utility in predicting PFS, irrespective of region and threshold value (p<0.05).

Conclusion: Results of the present meta-analysis revealed that an increased SIRI significantly predicted OS and PFS in patients diagnosed with PC. Considering its cost-effectiveness and availability, the SIRI may be a promising biomarker for predicting prognosis in patients with PC.

KEYWORDS

SIRI, pancreatic cancer, prognosis, evidence-based medicine, biomarker

Introduction

Pancreatic cancer (PC) ranks among the most common cancers of the digestive system and is characterized by poor prognosis and limited oncological treatment options (1). The global burden of PC has more than doubled in the past 25 years, ranking it as the seventh major cause of cancer-associated mortality globally (2). According to statistics from GLOBOCAN, 495,773 new cases of PC were diagnosed, with 466,003 related deaths reported worldwide in 2020 (2). Once detected, PC is usually in an advanced stage and cannot be surgically resected in approximately 80% of cases (3). There is only a 20% surgical resection rate in cases of PC that develop local or distant metastases, and metastasis and recurrence often occur even after surgical treatment (4). PC is highly malignant, difficult to diagnose early, and difficult to treat once it has already progressed (5). Pancreatic ductal adenocarcinoma (PDAC) is the most prevalent of PC subtypes, and is the deadliest malignancy, with a five-year survival rate < 8% (6). Consequently, the identification of novel and effective markers for individuals diagnosed with PC is urgently needed.

In recent years, inflammatory and immune responses have been suggested to play crucial roles in cancer progression and development (7). Many hematological parameters have been identified as significant prognostic markers for various cancers, such as lymphocyte-to-monocyte ratio (8), platelet-to-lymphocyte ratio (9), C-reactive protein-to-albumin ratio (10), controlling nutritional status score (CONUT) (11), and fibrinogen-to-albumin ratio (12). The systemic inflammation response index (SIRI) is calculated using neutrophil, lymphocyte, and monocyte counts (13). First proposed in 2016, the SIRI is calculated as neutrophil count × monocyte count/lymphocyte count (13). Recently, the SIRI was demonstrated to be highly significant in predicting the prognosis of various solid tumors, including non-small cell lung (14), breast (15), gastric (16), rectal (17), and hepatoblastoma (18) cancers. The SIRI has been widely analyzed for its prognostic significance in patients diagnosed with PC, although findings remain inconsistent (13, 19–24). As such, we performed a comprehensive literature review and meta-analysis to more precisely define the prognostic utility of the SIRI in patients diagnosed with PC.

Materials and methods

Study guideline

The current literature review and meta-analysis was performed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (i.e., "PRISMA") guidelines (25).

Abbreviations: SIRI, systemic inflammation response index; PC, pancreatic cancer; OS, overall survival; PFS, progression-free survival; HR, hazard ratio; CI, confidence interval; PDAC, pancreatic ductal adenocarcinoma; CONUT, controlling nutritional status score; PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses; NOS, Newcastle-Ottawa Scale; CRT, chemoradiotherapy; ROC, receiver operating characteristic.

Search strategy

A comprehensive search of the PubMed, Web of Science, Embase, and Cochrane Library databases for potentially eligible studies, published up to June 25, 2024, was performed using the following search terms: "systemic inflammation response index" or "systemic inflammatory response index" or "SIRI" and "pancreatic cancer" or "pancreatic carcinoma" or "pancreatic tumor" or "pancreatic adenocarcinoma" or "pancreatic neoplasm". Eligible studies were restricted to those published in English. Additionally, the reference lists of the retrieved studies were manually searched for other potentially eligible studies that fulfilled the inclusion criteria.

Inclusion and exclusion criteria

Studies fulfilling the following criteria were included: PC diagnosed by histological or cytological examination; reported the relationship between the SIRI and any survival of PC cases; relevant data including hazard ratio (HR) and 95% confidence interval (CI); identification of the threshold SIRI; and available full-text published in English. This meta-analysis utilized the pretreatment measured SIRI, excluding SIRI values assessed at various timepoints such as post-operative or pre/post neoadjuvant chemotherapy.

Case reports, meeting abstracts, letters, comments, and reviews, and studies with duplicate patients and animal studies were excluded.

Data collection and quality evaluation

Two researchers (HS and FZ) extracted data from the included studies, and disputes were resolved through consensus discussion. The following information was obtained from each included study: first author; publication year; country; sample size; age; sex; study duration; study center; study design; tumor stage; cancer type; threshold SIRI; threshold determination approach; survival outcomes; survival analysis types; survival endpoints; follow-up; and HRs with corresponding 95% CIs. Overall survival (OS) and progression-free survival (PFS) were the primary and secondary endpoints, respectively. Quality assessment was performed using the Newcastle–Ottawa Scale (NOS) (26). The NOS assesses study quality from 3 perspectives: selection, outcome, and comparability. The total NOS score ranges from 0 to 9, with scores ≥ 6 indicating high quality.

Statistical analysis

The utility/significance of the SIRI for predicting PC prognosis was estimated by calculating combined HRs and corresponding 95% CIs. Heterogeneity among the studies was evaluated using Cochran's test and the Higgins I^2 statistic. Results with $p \geq 0.10$ and $I^2 \leq 50\%$ represented no obvious heterogeneity and a fixed-effects model was used to analyze data; otherwise, a random-effects model was adopted. Subgroup analyses according to different factors were performed to detect the sources of heterogeneity for further

investigation. Stability of the results was evaluated using sensitivity analysis, in which each study was excluded one-at-a-time (i.e., "leave-one-out" method). Funnel plots were constructed and Begg's and Egger's tests were used to evaluate publication bias. Statistical analysis was performed using Stata Release 12.0 (StataCorp LLC, College Station, TX, USA). Differences with $p < 0.05$ were considered to be statistically significant.

Results

Search results

The initial literature search retrieved 399 studies, of which 298 were retained after duplicates were removed (Figure 1). After title and abstract review, 247 studies were excluded due to irrelevance and animal studies, and the full texts of 51 studies were further examined. Forty-four studies were excluded for the following reasons: irrelevance to the SIRI (n=38); meeting abstracts (n=2); lack of survival data (n=1); non-English publication (n=1); letters (n=1); and studies with duplicate patients (n=1). Ultimately, 7 studies comprising 1160 patients (13, 19–24) were included in the present meta-analysis (Figure 1; Table 1).

Study features

All enrolled studies were published between 2016 and 2024 (Table 1). Two were performed in China (13, 19) and 1 each in Turkey (20), Portugal (21), the United Kingdom (22), South Korea (23), and Spain (24). Sample sizes ranged from 50 to 371 (median, 152). There were 5 single-center studies (13, 19–21, 23) and 2 were multicenter trials (22, 24). Six studies had a retrospective design (13, 19–23) and 1 was a prospective trial (24). Four studies recruited patients with TNM stages III-IV (13, 20–22), 2 enrolled patients with stages I-III (19, 23), and 1 included patients with stage IV disease (24). Four studies treated patients with PC with chemotherapy (13, 21, 22, 24), and 1 each used surgery (19), chemoradiotherapy (CRT) (20), and neoadjuvant chemotherapy (NACT) + surgery (23). Five studies included patients with PDAC (19–23), and 2 included patients with PC (13, 24). The threshold SIRI was 0.69–2.35. All studies used receiver operating

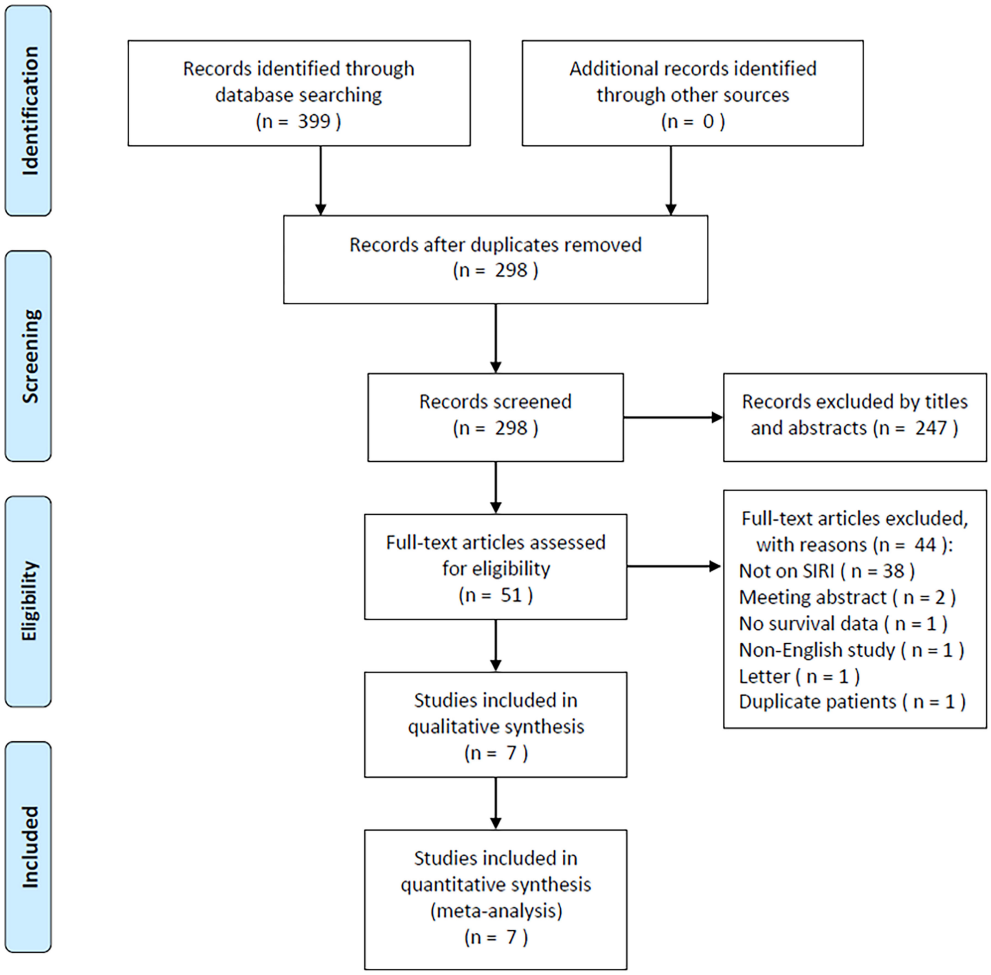

FIGURE 1
PRISMA flowchart explaining the article selection.

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TABLE 1 Basic characteristics of included studies in this meta-analysis..

Author	Year	Country	Sample size	Gender (M/F)	Age (years) Median (range)	Study period	Study center	Stude design	TNM stage	Treatment	Cancer type	SIRI cut-off value	Cut-off determination	Survival endpoints	Survival analysis	Follow-up (months) Median(range)	NOS score
Qi, Q.	2016	China	177	108/69	58.8	2009-2010	Single center	Retrospective	III-IV	Chemotherapy	PC	1.8	ROC curve	OS, PFS	Multivariate	1-10	8
Li, S.	2019	China	371	224/147	62(35-84)	2011-2013	Single center	Retrospective	I-III	Surgery	PDAC	0.69	ROC curve	OS, PFS	Multivariate	1-80	7
Topkan, E.	2021	Turkey	152	119/33	52(27-79)	2007-2019	Single center	Retrospective	III-IV	CRT	PDAC	1.8	ROC curve	OS, PFS	Multivariate	18.5(3.2-91.3)	8
Dâmaso, S.	2022	Portugal	112	53/59	71(34-88)	2016-2021	Single center	Retrospective	III-IV	Chemotherapy	PDAC	1.34	ROC curve	OS, PFS	Univariate	8.7(1-52)	7
Kamposioras, K.	2022	UK	138	87/51	62(29-77)	2010-2019	Multicenter	Retrospective	III-IV	Chemotherapy	PDAC	2.35	ROC curve	OS	Multivariate	47.2(0.3-64.9)	8
Kim, J. S.	2022	South Korea	160	92/68	61.8	2006-2019	Single center	Retrospective	I-III	NACT + surgery	PDAC	0.95	ROC curve	OS, PFS	Univariate	30(1-140)	8
Pacheco-Barcia, V.	2024	Spain	50	32/18	66(32-85)	2020-2023	Multicenter	Prospective	IV	Chemotherapy	PC	2.3	ROC curve	OS, PFS	Univariate	1-48	9

M, male; F, female; SIRI, systemic inflammation response index; OS, overall survival; PFS, progression-free survival; ROC, receiver operating characteristic; CRT, chemoradiotherapy; PDAC, pancreatic ductal adenocarcinoma; NACT, neoadjuvant chemotherapy; TNM, tumor-node-metastasis; NOS, Newcastle-Ottawa Scale.

characteristic (ROC) curve analysis to determine threshold values. All 7 included studies reported the relationship between the SIRI and OS (13, 19–24), whereas 6 presented the significance of the SIRI in predicting PFS (13, 19–21, 23, 24) in PC. Three studies obtained HRs and 95% CIs using univariate regression (21, 23, 24), while 4 used multivariate regression (13, 19, 20, 22). NOS scores ranged from 7 to 9, suggesting high quality (Table 1).

SIRI and OS in PC

Seven studies involving 1160 patients (13, 19–24) reported data regarding the relationship between the SIRI and OS in PC. Due to obvious heterogeneity (I^2 = 69.7%, p = 0.003), a random-effects model yielded an HR of 2.40 (95% CI 1.88–3.05; p < 0.001), suggesting that an elevated SIRI was the significant prognostic marker for OS in patients with PC (Figure 2; Table 2). As demonstrated by subgroup analysis, the SIRI remained a significant predictor of OS regardless of region, sample size, study center, study design, TNM stage, cancer type, cut-off value, treatment, or survival analysis type (all p < 0.05) (Table 2).

SIRI and PFS of PC

Six studies comprising 1022 patients (13, 19–21, 23, 24) reported SIRI values for predicting PFS in patients diagnosed with PC. Due to significant heterogeneity (I^2 = 93.1%, p < 0.001), a random-effects model was used. Based on pooled data, a higher SIRI was markedly associated with dismal PFS in patients with PC (HR 1.95 [95% CI 1.19–3.21]); p = 0.008) (Figure 3; Table 3). Based on subgroup analysis, the significant prognostic value of the SIRI for PFS remained unaffected by region or cut-off value (p<0.05) (Table 3). Additionally, the SIRI still significantly predicted PFS of PC in the following subgroups: sample size ≥ 150 (p = 0.021); multicenter studies (p < 0.001); prospective studies (p < 0.001); TNM stage I-III (p = 0.002) and stage IV (p < 0.001); PDAC histology (p = 0.037); treatment of surgery/NACT+ surgery (p = 0.002) and CRT (p < 0.001); and multivariate survival analysis (p = 0.028) (Table 3).

Sensitivity analysis

Results of sensitivity analyses using the "leave-one-out" method for OS and PFS are reported in Figure 4. One study did not demonstrate significant changes in OS or PFS in this meta-analysis, indicating that the findings were reliable (Figure 4).

Publication bias

Funnel plots and Egger's and Begg's tests were used to estimate possible publication bias. Publication bias was not detected for OS (p = 1.000 and p = 0.305 according to Begg's and Egger's tests, respectively) and PFS (p = 0.707 and p = 0.060 according to Begg's and Egger's tests, respectively) (Figure 5).

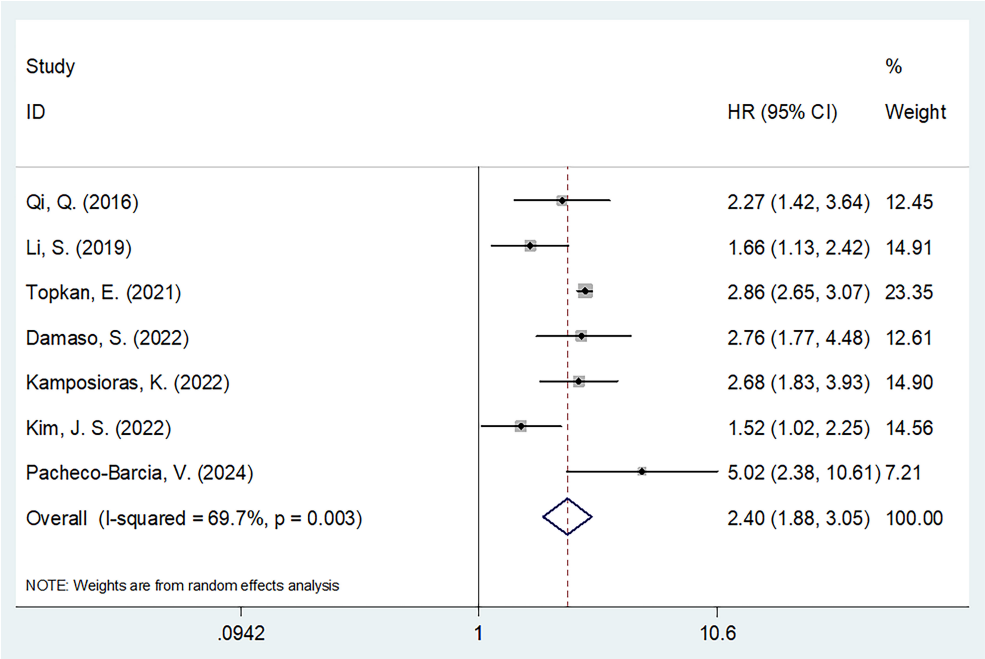

FIGURE 2
Forest plots of the association between SIRI and overall survival in patients with PC.

TABLE 2 Subgroup analysis of prognostic value of SIRI for OS in patients with pancreatic cancer.

Subgroups	No. of studies	No. of patients	Effects model	HR (95%CI)	p	Heterogeneity	
						I^2(%)	Ph
Total	7	1160	Random	2.40(1.88-3.05)	<0.001	69.7	0.003
Region							
Asian	3	708	Fixed	1.74(1.37-2.70)	<0.001	0	0.414
Non-Asian	4	452	Fixed	2.87(2.67-3.08)	<0.001	0	0.512
Sample size							
<150	3	300	Fixed	2.94(2.24-3.88)	<0.001	11.6	0.323
≥150	4	860	Random	2.06(1.42-2.98)	<0.001	82.6	0.001
Study center							
Single center	5	972	Random	2.18(1.61-2.94)	<0.001	78.6	0.002
Multicenter	2	188	Random	3.37(1.86-6.08)	<0.001	53.3	0.143
Study design							
Retrospective	6	1110	Random	2.27(1.77-2.90)	<0.001	71.1	0.004
Prospective	1	50	–	5.02(2.37-10.61)	<0.001	–	–
TNM stage							
I-III	2	531	Fixed	1.59(1.21-2.09)	0.001	0	0.756
III-IV	4	579	Fixed	2.84(2.64-3.04)	<0.001	0	0.803
IV	1	50	–	5.02(2.37-10.61)	<0.001	–	–

(Continued)

TABLE 2 Continued

Subgroups	No. of studies	No. of patients	Effects model	HR (95%CI)	p	Heterogeneity	
						I^2(%)	Ph
Cancer type							
PC	2	227	Random	3.20(1.48-6.89)	0.003	67.5	0.079
PDAC	5	933	Random	2.26(1.70-2.99)	<0.001	76.0	0.002
SIRI cut-off value							
<1.8	3	643	Random	1.86(1.33-2.62)	<0.001	51.3	0.129
≥1.8	4	517	Fixed	2.85(2.66-3.06)	<0.001	5.9	0.364
Treatment							
Chemotherapy	4	477	Fixed	2.76(2.17-3.50)	<0.001	4.0	0.373
Surgery/ NACT+ surgery	2	531	Fixed	1.59(1.21-2.09)	0.001	0	0.756
CRT	1	152	–	2.86(2.66-3.08)	<0.001	–	–
Survival analysis							
Univariate	3	322	Random	2.59(1.38-4.87)	0.003	77.7	0.011
Multivariate	4	838	Random	2.41(1.87-3.11)	<0.001	64.2	0.039

SIRI, systemic inflammation response index; CRT, chemoradiotherapy; PDAC, pancreatic ductal adenocarcinoma; PC, pancreatic cancer; NACT, neoadjuvant chemotherapy; TNM, tumor-node-metastasis.

Discussion

The efficiency of the SIRI in predicting PC prognosis has been extensively analyzed; however, findings have been inconsistent. For example, a high SIRI has been suggested to be a significant prognostic marker of PC in some studies (13, 19, 20, 22–24). In contrast, some clinicians have failed to determine a relationship between the SIRI and PC prognosis (21). These inconsistencies prevent the clinical application of SIRI for PC prognostication.

In this meta-analysis, we aggregated data from 7 studies involving 1160 patients (13, 19–24) to more clearly define the prognostic utility of the SIRI. Based on our results, a higher SIRI

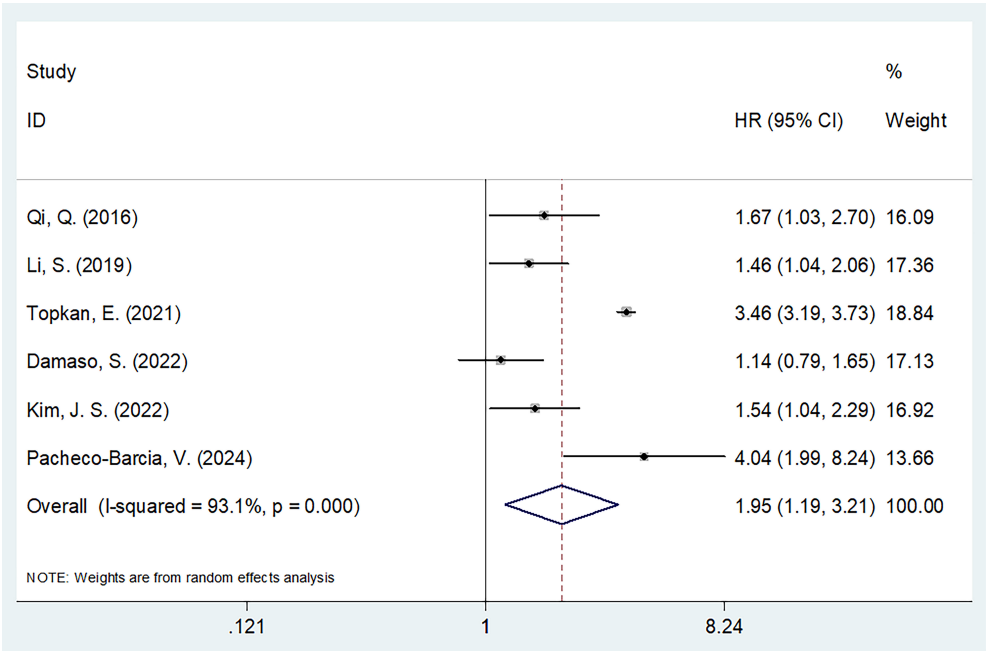

FIGURE 3
Forest plots of the association between SIRI and progression-free survival in patients with PC.

TABLE 3 Subgroup analysis of prognostic value of SIRI for PFS in patients with pancreatic cancer.

Subgroups	No. of studies	No. of patients	Effects model	HR (95%CI)	p	Heterogeneity	
						I^2(%)	Ph
Total	6	1022	Random	1.95(1.19-3.21)	0.008	93.1	<0.001
Region							
Asian	3	708	Fixed	1.53(1.22-1.93)	<0.001	0	0.905
Non-Asian	3	314	Random	2.47(1.10-5.55)	<0.001	94.0	<0.001
Sample size							
<150	2	162	Random	2.07(0.60-7.12)	0.250	89.5	0.002
≥150	4	860	Random	1.94(1.1-3.40)	0.021	93.2	<0.001
Study center							
Single center	5	972	Random	1.74(1.00-3.03)	0.050	94.5	<0.001
Multicenter	1	50	–	4.04(1.99-8.22)	<0.001	–	–
Study design							
Retrospective	5	972	Random	1.74(1.00-3.03)	0.050	94.5	<0.001
Prospective	1	50	–	4.04(1.99-8.22)	<0.001	–	–
TNM stage							
I-III	2	531	Fixed	1.50(1.15-1.94)	0.002	0	0.846
III-IV	3	441	Random	1.91(0.87-4.20)	0.108	95.1	<0.001
IV	1	50	–	4.04(1.99-8.22)	<0.001	–	–
Cancer type							
PC	2	227	Random	2.50(1.06-5.90)	0.037	75.4	0.044
PDAC	4	795	Random	1.75(0.92-3.33)	0.086	95.5	<0.001
SIRI cut-off value							
<1.8	3	643	Fixed	1.37(1.11-1.69)	0.004	0	0.492
≥1.8	3	379	Random	2.85(1.73-4.69)	<0.001	77.3	0.012
Treatment							
Chemotherapy	3	339	Random	1.86(0.98-3.52)	0.059	79.4	0.008
Surgery/ NACT+ surgery	2	531	Fixed	1.50(1.15-1.94)	0.002	0	0.846
CRT	1	152	–	3.46(3.20-3.74)	<0.001	–	–
Survival analysis							
Univariate	3	322	Random	1.78(0.99-3.22)	0.055	79.1	0.008
Multivariate	3	700	Random	2.08(1.08-4.02)	0.028	93.4	<0.001

SIRI, systemic inflammation response index; CRT, chemoradiotherapy; PDAC, pancreatic ductal adenocarcinoma; PC, pancreatic cancer; NACT, neoadjuvant chemotherapy; TNM, tumor-node-metastasis.

significantly predicted OS and PFS in patients with PC. Moreover, the role of the SIRI in predicting OS and PFS remained unaffected by geographical region and cut-off values in PC. As verified by publication bias and sensitivity analyses, our findings were stable. Collectively, a higher SIRI significantly predicted the short- and long-term prognoses of patients with PC. The SIRI may a candidate biomarker for predicting PC prognosis due to its cost-effectiveness and availability. To the best of our knowledge, this meta-analysis is the first to explore the utility of the SIRI in predicting PC prognosis.

We computed the SIRI using neutrophil, lymphocyte, and monocyte counts. Consequently, a higher SIRI may be due to higher neutrophil/monocyte counts and/or lower lymphocyte counts. Although the precise mechanisms underlying the significance of the SIRI in predicting PC prognosis remain largely unclear, they are interpreted as follows. First, it

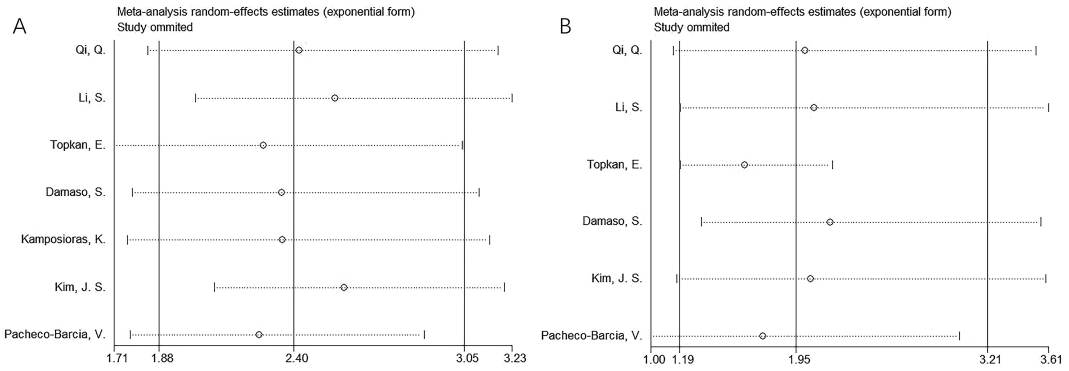

FIGURE 4
Sensitivity analysis. **(A)** OS; and **(B)** PFS.

is known that neutrophils produce growth factors, chemokines and cytokines that can promote angiogenesis, such as transforming growth factor-beta, vascular endothelial growth factor, matrix metalloproteinases, and interleukin (IL)-6, IL-8, and IL-12 (27). In addition to secreting cytokines, neutrophils also produce proteases, including matrix metalloproteinases, cysteine cathepsins, and serine proteases (28, 29). Because these proteases disrupt cell connections and degrade the extracellular matrix and basement membrane proteins, tumor cells can migrate more easily (30). Second, monocytes may affect tumor occurrence by differentiating into tumor-associated macrophages (TAMs). Chemokines and cytokines in the tumor microenvironment exert a chemotactic effect on TAMs, including tumor necrosis factor-α and monocyte chemoattractant protein-1, among others (31). Furthermore, monocytes can inhibit antigen- and mitogen-induced lymphocyte proliferation, impair lymphocyte-dependent antitumor defenses, and suppress antitumor immunity (32). Third, lymphocytes, particularly tumor-infiltrating lymphocytes (TILs), are important for cell-mediated immunity against tumors (33). Lower lymphocyte counts can weaken the systemic immune system; therefore, tumor cells can evade immune surveillance, ultimately enhancing their malignant behavior (34).

Results of the present meta-analysis have important implications for clinical practice. First, variations in the follow-up duration of the included studies may have affected the prognostic role of the SIRI.

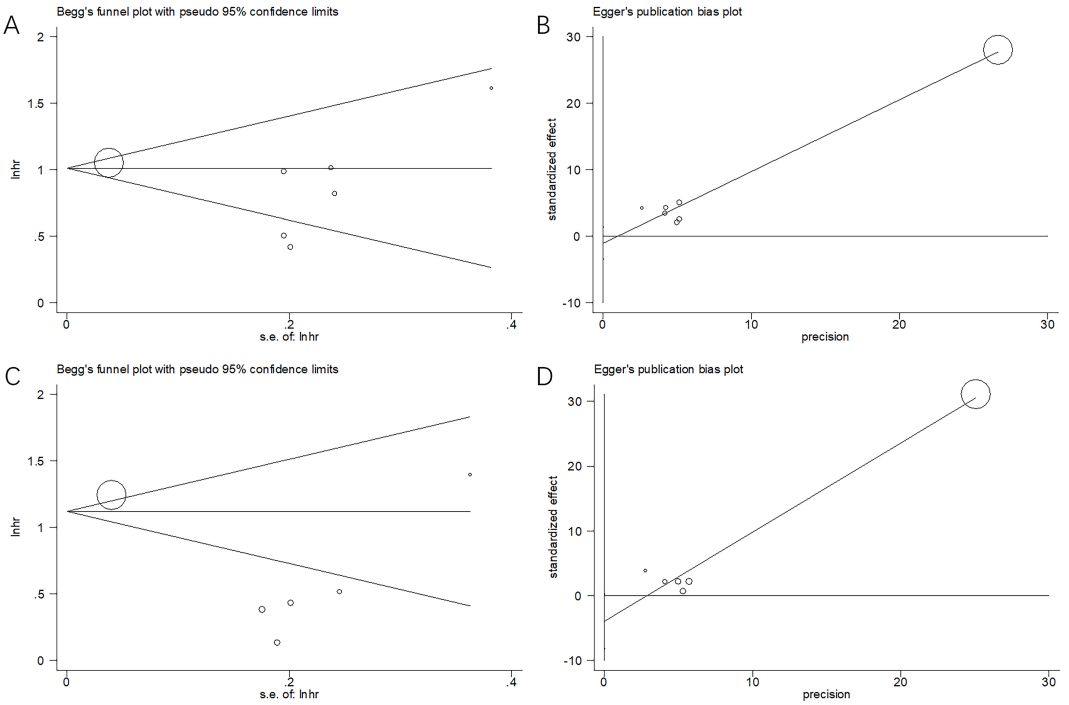

FIGURE 5
Publication bias test. **(A)** Begg's test for OS, p=1.000; **(B)** Egger's test for OS, p=0.305; **(C)** Begg's test for PFS, p=0.707; and **(D)** Egger's test for PFS, p=0.060.

Therefore, adequate follow-up is needed for the post-treatment management of PC. Second, the SIRI may vary during the treatment process for PC. In this meta-analysis, we adopted pretreatment blood test parameters to calculate the SIRI. Moreover, infections, trauma, and immune-related diseases should be excluded when the SIRI is calculated because they can affect specific immunological indices. Third, this meta-analysis included only the pretreatment SIRI. Changes in SIRI scores before and after treatment may provide prognostic value, which should be explored in future studies. Subgroup analysis indicated that an increased SIRI was significantly associated with poor OS and PFS in patients with PC who underwent surgery or NACT + surgery. However, an elevated SIRI was a significant prognostic marker for poor OS——but not PFS——in patients with PC treated with chemotherapy. Therefore, in patients with resectable PC, the SIRI remains a significant prognostic indicator of both OS and PFS.

Notably, SIRI cut-off values varied among the included studies, ranging from 0.69 to 2.35, with a median value of 1.8; as such, 1.8 was adopted as the cut-off value in the subgroup analysis. The carbohydrate antigen 19-9 (CA 19-9) is a glycoprotein found on the cell surface of the pancreatic ductal cells (35). A wide range of benign diseases, such as cholestasis, and malignant diseases, mainly PDAC, overexpress CA19-9 (36). Preoperative serum levels of CA 19-9 are associated with both occult metastasis detection during surgery and worse disease-free survival (DFS), even in resectable PDAC (37). For patients with PDAC, CA 19-9 is considered to be the main biological parameter to assess its biological resectability (38). Whether the combination of SIRI and CA19-9 could enhance the prognostic efficiency for PDAC patients is needed to be investigated in future studies.

Recently, SIRI is widely suggested with prognostic significance for different cancer types by meta-analysis (39–43). As reported by Zhang et al. (39), a higher SIRI estimated poor OS and PFS in hepatocellular carcinoma cases in a meta-analysis of 10 studies. Ren et al. (41) conducted a meta-analysis of 6 studies and found that a higher SIRI value was consistently related to poor OS and DFS in patients with gastric cancer. In addition, another meta-analysis enrolling 3187 patients reported that the SIRI independently predicted OS in nasopharyngeal carcinoma (42). In a recent meta-analysis involving 2997 cases, a higher SIRI was markedly related to poor OS but not poor DFS in breast cancer (40). Our meta-analysis is consistent with results regarding the prognostic utility of the SIRI in other cancer types.

However, the present study had several limitations, the first of which was the small sample size, with only 7 studies included. Second, most included studies were retrospective in design, which may have introduced selection bias due to the inherent nature of such designs. Third, the threshold SIRI was not uniform among the included studies, which may have contributed to heterogeneity. Fourth, it is important to note that many non-specific biological processes may affect the cell counts necessary to calculate SIRI (pathology, cancer, infection, inflammation, etc.). Given these limitations, large-scale prospective studies should be conducted for further validation.

Conclusions

In summary, results of the present meta-analysis demonstrated that an elevated SIRI significantly predicted OS and PFS in patients diagnosed with PC. Considering its cost-effectiveness and availability, the SIRI may be a promising prognostic biomarker in this patient population.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author/s.

Author contributions

HS: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft. FZ: Conceptualization, Data curation, Investigation, Methodology, Project administration, Software, Validation, Visualization, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Prolonged survival by combination treatment with a standardized herbal extract from Japanese Kampo-medicine (Juzentaihoto) and gemcitabine in an orthotopic transplantation pancreatic cancer model

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Pancreatic ductal adenocarcinoma (PDAC) is characterized by its poor prognosis. Traditional Japanese herbal medicine (Kampo), such as Juzentaihoto (a standardized combination of 10 herbal extracts), has shown immune modulatory effects, modulation of microcirculation, and amelioration of fatigue. It is administered to patients to prevent deterioration of cachexia and counteract side effects of chemotherapy. The effect of Juzentaihoto with or without standard chemotherapy (Gemcitabine) on survival and tumor microenvironment was studied in an immunocompetent pancreatic cancer mouse model. Following tumor development ±12 days after orthotopic implantation of murine pancreatic cancer cells (KPC) into the pancreas of C57BL/6 mice, the mice were treated with Gemcitabine, Juzentaihoto, their combination (Gem/Juz) or NaCl (Ctr.). Combination treatment significantly prolonged survival (+38%) of tumor bearing mice, compared to controls as well as Gemcitabine or Juzentaihoto monotherapy. Macrophage (CD68+) infiltration in pancreatic tumors was significantly enhanced in Gem/Juz – treated animals, compared with controls ($p < 0,001$), with significant increases of both, macrophages (CD68+) and for lymphocytes (CD45+), especially at the tumor front. *In vitro*, Juz- or Gem/Juz-treated KPC tumor cells secreted significantly more macrophage-chemoattractant cytokines, e.g., CCL2, CCL20, and CXCL2, whilst Juz- and Gem/Juz-treated macrophages (MH-S) secreted cytokines of the M1 phenotype, e.g., IL6, TNF-α, and IL12. It has been shown that tumor cells recruit and polarize macrophages towards tumor-associated macrophages (TAM). Our results indicate a change in macrophage polarization which not only induced anti-tumor immune-cell activity and cytokine release, but

also suggests amelioration of Gemcitabine efficacy as DNA-analogue and as partial antitumor antigen. We propose that the increased survival of tumor bearing mice after Gem/Juz combination treatment is due to the restored cytotoxicity of Gemcitabine and changes in the tumor-microenvironment - induced by Juzentaihoto - such as an increased number of M1 macrophages.

KEYWORDS

Kampo medicine, Juzentaihoto, gemcitabine, pancreatic ductal adenocarcinoma (PDAC), orthotopic transplantation PDAC mouse model, murine KPC pancreatic tumor cells, macrophages, tumor microenvironment

1 Introduction

Pancreatic adenocarcinoma (PDAC) is characterized by an aggressive phenotype and an extremely poor prognosis. Due to the frequently asymptomatic onset of the disease, patients are usually presenting with locally advanced - or metastatic disease with less than 20% eligibility for initial resection. Most patients experience local or systemic disease recurrence, resulting in an overall 5-year survival rate of less than 9% (1). Partial resistance to chemotherapeutic drugs and/or radiation therapy is common (2).

Chronic inflammatory processes and microcirculatory abnormalities accompany the development and growth of neoplastic tissue (3). This leads to a dense tumor stroma consisting of extracellular matrix (ECM) and cellular components such as cancer-associated fibroblasts (CAF), immune cells, especially macrophages, and endothelial cells. Tumor-associated macrophages (TAM) engage in bidirectional interactions with cancer cells. TAM are highly versatile and can be polarized into the M1-like pro-inflammatory phenotype that activates an immune response against the tumor and the M2-like immunosuppressive phenotype that promotes tumor immunity and progression (4, 5). M1/M2 phenotyping represents, however, only two extremes of the observed macrophage profiles (5).

Balancing inflammatory and redox-mechanisms, amelioration of microcirculation as well as wound-healing and nutrition have always been indications for herbal medicine (6). Once the body constitution deteriorates and cachexia sets in, a point of no return has been reached, forcing dose reduction or even termination of therapy (7). Learning how to manage these accompanying phenomena could significantly improve patients' outcomes.

In Japan, traditional herbal medicine, Kampo, has been integrated into the modern medical system. Traditional prescriptions are standardized and controlled using GMP- and GCP-guidelines. Pharmaceutical and medicinal research is performed on an academic level and by pharmaceutical companies, besides trained clinical use. Together with the long-term experience over centuries, Kampo medicines – such as Juzentaihoto - can be used safely alongside Western medicine.

Juzentaihoto is covered by the Japanese National Health insurance and administered to support anti-tumor therapy, to alleviate side effects and in palliative cancer care to improve body constitution and appetite. This is particularly beneficial for patients with gastrointestinal cancers (8–10) and in particular for pancreatic cancer (6, 11–16). In China, this ancient Kampo-preparation is called *Shi-Quan-Da-Bu-Tang* and in Korea, *Sipjeondaebotang*.

Anti-cachexia are based - amongst others - on the activity of combined herbal extracts such as ginseng (*Panax ginseng*) or glycyrrhizae (*Glycyrrhiza uralensis*) radix on the mTOR-pathway, the STAT-pathway and melatonin (6) as well as ghrelin-enhancing properties (7).

As pancreatic cancer is the most aggressive gastrointestinal tumor, standardized Kampo extracts, especially Juzentaihoto with its immunomodulatory effects are of interest alongside chemotherapy (6, 7, 11, 17). It has been shown that Juzentaihoto increased leucocyte cell counts after chemotherapy (11) whilst protecting against myelosuppression (18). It also activated peritoneal macrophages against tumor cells and prevented liver- or lung metastasis (19, 20) in cell culture. The immune-enhancing effect of Juzentaihoto is involved in the prevention of metastasis, as this effect was abolished in mice with T-cell deficiencies (20).

Here, we show that the combination of Gemcitabine-based chemotherapy with the traditional herbal prescription Juzentaihoto prolonged the survival of pancreatic tumor-bearing mice. As the decrease of tumor size by Gemcitabine monotherapy did not translate into prolonged survival, we propose that Juzentaihoto-induced activation of tumor-associated macrophages not only induced anti-tumor immune-cell activity and cytokine release but also ameliorated Gemcitabine efficacy.

2 Materials and methods

2.1 Juzentaihoto extracts

Juzentaihoto consists of: *Panax ginseng* radix 3 g, *Atractylodes lancea* rhizome 4 g, *Poria cocos* sclerotium 4 g, *Glycyrrhiza uralensis* radix 2 g, *Angelica acutiloba* radix 4 g, *Paeonia lactiflora* radix 3 g, *Cnidium officinale* rhizome 3 g, *Rehmannia glutinosa* radix 4 g,

Astragalus membranaceus radix 3 g, and *Cinnamomum cassia* cortex 3 g (Supplementary Figure S1A) (21). The single herbs have been imported from Toshimoto, Japan and provided by the "Kronen-Pharmacy,Wuppertal".

For animal experiments, aqueous extracts of Juzentaihoto were freshly prepared from the above-named mixture of raw drugs: They were boiled for 30 min in water to a final volume of 400 ml shortly before use. For mice treatment, the final extracts were cooled down, filtered using common paper tea filter bags and changed 3 times per week to drink ad libitum. Whilst the leading components of Juzentaihoto are well characterized by HPLC (22), its efficacy relies on the combination of its multiple active ingredients such as components from Paeonia Radix (Paeoniflorin, and Albiflorin), Glycyrrhizae Radix (Isoliquritin, Liquiritin, Isoliquiritigenin, Glycyrrhizin and Formononetin), Cinnamomi Cortex (Cinnamaldehyde), and i.e. Angelicae Radix (Ligustilide and Xanthotoxin). Immunpharmacologically active polysaccharides have also been described, including pectins and pectic polysaccharides (23). In our experiments, we analyzed the combined effects, following the traditional application of Juzentaihoto.

For cell-culture experiments, the aqueous extract was lyophilized and re-diluted in cell-culture medium with 1% DMSO.

The quality of the extract was analyzed by HPLC (Supplementary Figures S1B, C). In brief: The aqueous extract of Juzentaihoto was filtered and used in comparison to the reference substance of the corresponding herbal drugs. Of each solution 125 µl were injected. For visual control, UV-spectra were employed. The HPLC from Merck-Hitachi (7000 D) was used with a corresponding HPLC-column [Merck (250 x 4 mm) type Lichrospher (100 RP18 5 µm)] and a precolumn (RP18 4 × 4 mm). The detection was performed by a diode array (210 nm) with a flow rate of 1 ml/min and a temperature of 20°C. Recording was done for about 100 min. The mobile phase contained acetonitril (0.05%), phorsphoric acid (1.5 ml + 2.5 ml aqua dest.) and methanol. The size of the membrane filter was 0.45 µm (Ø 3 mm, Schleicher & Schüll) with a Chromafil® GF/PER-45/25 Macherey nail (1.0/0.45 µm Ø 25mm), and with a syringe filter of 0.45 µm (Ø 4 mm) for single use.

2.2 Cell lines and cell culture conditions

The murine pancreatic cancer KPC cell line (KPCbl6, clone 2.2) derived from the LSL-KrasG12D/+; LSL-Trp53R172H/+; Pdx-1-Cre (KPC) mouse model (24) was provided by Prof. V. Ellenrieder (University Medicine Göttingen). KPC cells were cultivated in high glucose Dulbecco's modified Eagles medium (DMEM, Thermo Fisher Scientific, MA, Waltham, USA), supplemented with 10% fetal bovine serum (FBS Gold, PAA Laboratories Gold) and 1% non-essential amino acids (NEAA-100 X, Thermo Fisher Scientific, MA, Waltham, USA).

The murine alveolar macrophage MH-S cell line (CRL-2019, ATCC) was cultivated in complete Roswell Park Memorial Institute medium (RPMI, Thermo Fisher Scientific, MA, Waltham, USA) 1640, supplemented with 10% fetal calf serum (FCS) (Gibco) and 0.05 mM 2-mercaptoethanol (25).

All cells were maintained in culture at 37°C in a humidified atmosphere of 5% CO2.

2.3 Treatment efficacy *in vitro*

KPC cells were plated in a 96-well plate at a concentration of 5,000 cells per well and allowed to attach for ~24 h.

Afterwards, the medium was replaced with 100 µl of fresh medium supplemented with increasing concentrations of either Gemcitabine (HEXAL; 0, 1, 5, 10, 20, 40, 60, 80, 100, 125, 150, and 200 nM), or Juzentaihoto (water extract diluted 1:10, 1:20, 1:50, 1:75, 1:100, 1:200, 1:300, 1:400, 1:500, and 1:1000 in cell culture medium supplemented with 1% dimethyl sulfoxide (DMSO) or with a combination of both. Control cells received corresponding volumes of water and 1% DMSO. The experiment was performed in duplicate.

Plates were placed in the Incucyte Live-Cell Imaging and Analysis Instrument (Sartorius). Phase contrast images (two images per well) were collected every hour with a 10× objective. The IC50 values were calculated after 72 h of incubation - a time when untreated cells reached 100% confluence.

2.4 RNA extraction and real-time PCR

KPC and MH-S cells were plated in a 6-well plate at a concentration of 1×10^6 cells per well (or 0.5×10^6 cells per well, respectively, and allowed to attach for ~24 h. Afterwards, medium was replaced with 2 ml of fresh medium supplemented with either Gemcitabine (HEXAL; 80 nM), or Juzentaihoto (water extract diluted 1:20 in cell culture medium supplemented with 1% DMSO or a combination of both. Water (1:20) and DMSO (1%) were used as controls.

After 24 h, the cells were washed with phosphate buffered saline (PBS), and total RNA was extracted with the TRIzol® reagent (Thermo Fisher Scientific, MA, Waltham, USA). After addition of 200 µl chloroform, the tubes were vortexed and incubated at room temperature for 5 min. After a centrifugation step at 13,500 rpm at 4°C for 15 min, the supernatant was separated and 500 µl isopropanol was added. After 10 min incubation at room temperature probes were centrifuged (13,500 rpm at 4°C) for 30 min. The pellet was then diluted in 750 µl 75% ethanol and vortexed. After 5 min centrifugation (13,500 rpm at 4°C), the supernatant was discharged and a second washing step with 75% EtOH was performed, followed by centrifugation. The pellet was then dried and 30 µl of RNA-free water was added on ice.

For cDNA synthesis the iScript cDNA Synthesis Kit was used according to the manufacturer's instructions (Bio-Rad Laboratories, Feldkirchen, Germany), with the following program: 25°C (5 min), 46°C (20 min), 95°C (1 min), then cooling to 4°C. After adding 80 µl, RNA-free water cDNA was stored at -20°C.

Quantification of the mRNA was performed by relative quantification using Platinum™ SYBR™ Green qPCR SuperMix-UDG (Invitrogen, Darmstadt, Germany) in the following concentrations: 5 µl SYBR Green, 3.5 µl H2O dest., 0.25 µl

forward primer, and 0.25 µL reverse primer. Then, 1 µl cDNA was added and RT-PCR (96 well-plate) was performed in triplicates. Supplementary Table S1 shows the list of the primers used (Invitrogen, Darmstadt, Germany).

PCR conditions were set as follows: 50°C for 2 min, 95°C for 2 min, and 45 cycles of 95°C/15 sec and 60°C/30 sec. As housekeeping gene, Ribosomal Protein, Large, P0 (RPLP0) was used, which was checked for stability. For data analysis, Step one PluS software (v2.3) was employed.

2.5 Cytokine/chemokine array

For treatment experiments, KPC and MH-S cells were plated at a concentration of 1×10^6 or 0.5×10^6 cells per well, respectively, in a 6-well plate and allowed to attach for ~24 h. Afterwards, the medium was replaced with 2 ml of fresh medium supplemented with either Gemcitabine (HEXAL; 80 nM), Juzentaihoto (water extract diluted 1:20 in cell culture medium supplemented with 1% DMSO) or a combination of both. Medium with addition of water (1:20) and DMSO (1%) was used as control. After 24 h treatment, supernatants were collected, centrifuged for 5 min at 14.000 rpm, stored at -20°C and used for cytokine profiling.

Cytokine profiling was performed using the Mouse XL Cytokine Array Kit (Proteome ProfilerTM Array; R&D systems) with 1 ml of cell culture supernatants (KPC and MH-S cells), according to the manufacturers protocol. Data were quantified using ImageJ software (FIJI) (26) and are shown as an average intensity normalized to the reference spots after background subtraction.

2.6 Vimentin-cadherin immunofluorescence staining

Aliquots of 50,000 cells/well were grown on poly-L-lysine coated coverslips in a 24-well plate for 24 h, followed by a 24 h treatment with Gemcitabine, or Juzentaihoto, or a combination of both, or water/DMSO as a control as described above in the Cytokine/chemokine array section. Afterwards, the cells were washed briefly with PBS, fixed with ice-cold 4% formaldehyde for 15 min, washed again with PBS (3 × 10 min), and permeabilized in 1% Triton X-100 in PBS for 10 min followed by three PBS washes. Subsequently, the cells were blocked in 0.5% bovine serum albumin (BSA) in PBS for 1 h at room temperature (RT) and incubated with the mouse monoclonal anti-vimentin antibody (Santa Cruz Biotechnology, E-5, 1:200) and the rabbit monoclonal anti-E-cadherin antibody (Cell signaling, 24E10, 1:100) in 0.5% BSA for 1 h at RT. After three washes with 0.1% Tween20 in PBS for 10 min each, the cells were incubated for 1 h at RT with fluorescence-labeled secondary antibodies: anti-mouse (Thermo Fisher, A-11003, Alexa Fluor 546) and anti-rabbit (Thermo Fisher, A-21206, Alexa Fluor 488), each diluted 1:500 in 0.5% BSA in PBS. Nuclei were stained for 10 min with Hoechst 33342 diluted 1:1.000 in PBS. The cover slips were mounted with Aquatex aqueous mounting medium (Merck). Confocal fluorescence microscopy was performed using the Leica SP2 system. The following excitation and emission settings were used: for Alexa Fluor 488: λEx = 488 nm and λEm = 500-550 nm, for Alexa Fluor 546: λEx = 561 nm und λEm = 570-650 nm and for DAPI: λEx = 405 nm and λEm= 410-480 nm. Images were analyzed using ImageJ software (FIJI) (26).

2.7 Animal studies

All animal experiments were performed in accordance with German Animal Welfare Act regulations and were approved by the Local Ethics Office of Lower Saxony (LAVES; license no. 33.9-42502-04-18/2953; Oldenburg, 22.11.2018). Experiments were performed on 10-16 weeks old C57BL/6 male mice (Charles River Laboratories), housed in ventilated cages, and allowed food and drink (water or Juzentaihoto) ad libitum.

30-45 min prior to the surgery, mice received analgesic buprenorphine (intraperitoneally, 0.1 mg/kg body weight (BW); 10 µl/g BW). After induction of anesthesia with isoflurane (4%, maintenance 2.5%), mice received the analgesic Rimadyl® (subcutaneously; Carprofen 5 mg/kg BW, 5 µl/g BW, Zentiva) and the abdominal skin and peritoneum were opened with a ~5 mm incision in the left upper abdomen. The pancreas was exposed and ~1.8×10^5 cells resuspended in 40 µl of culture medium/matrigel (1:1) were slowly transplanted into the pancreatic tail with an insulin syringe. The pancreas was reintroduced into the abdominal cavity and peritoneum and skin were sutured. For analgesia, mice received Rimadyl for 2 days after surgery. Mice were weighted and inspected three times per week for general condition, and abdominal palpation was performed to detect tumor formation. Tumor size was measured weekly with small animal high-resolution ultrasound as previously described (27). Briefly, isoflurane (~2%) anesthetized mice were placed on a heated stage and the abdominal area was depilated. Ultrasonography (US) was performed using the Visual Sonics Vevo 2100 High Resolution Ultrasound System equipped with the Vevo 2100 MicroScan Transducer MS-550-D (22-55 MHz). Sagittal and transversal planes were imaged (Figures 1A–C).

After development of visible (US) pancreatic tumor (day 12), mice were randomized into four groups: placebo (n = 6), Gemcitabine (Gem) (n = 6), Juzentaihoto (Juz) (n = 9), and Gemcitabine plus Juzentaihoto (Gem/Juz) (n = 9). Fresh Juzentaihoto decoction was prepared 3 times per week as described above and administered ad libitum in place of drinking water. Gemcitabine (HEXAL) was diluted in 0.9% NaCl (100 mg/kg BW; 5µl/g BW) and administered intraperitoneally (i.p.) three times per week. Placebo-treated mice received equal volumes of saline injections (NaCl 0.9%). Treatment was continued until each individual animal reached the defined endpoint. Endpoint criteria were defined as 20% BW loss, general morbidity, lethargy, lack of social interaction or development of ascites. Kaplan-Meier survival analysis was performed using GraphPad Prism (9.5.0).

During autopsy (Figure 1D), each tumor was measured with a caliper and tumor volume was calculated based on a simplified formula: length × width × high × 0.5. Tumor and remaining pancreas were collected, fixed in 4% paraformaldehyde (PFA, Sigma) for 24 h, dehydrated in ascending concentrations of ethanol and embedded in paraffin. Tissue sections 2.5 µm were processed for histochemistry, i.e.,

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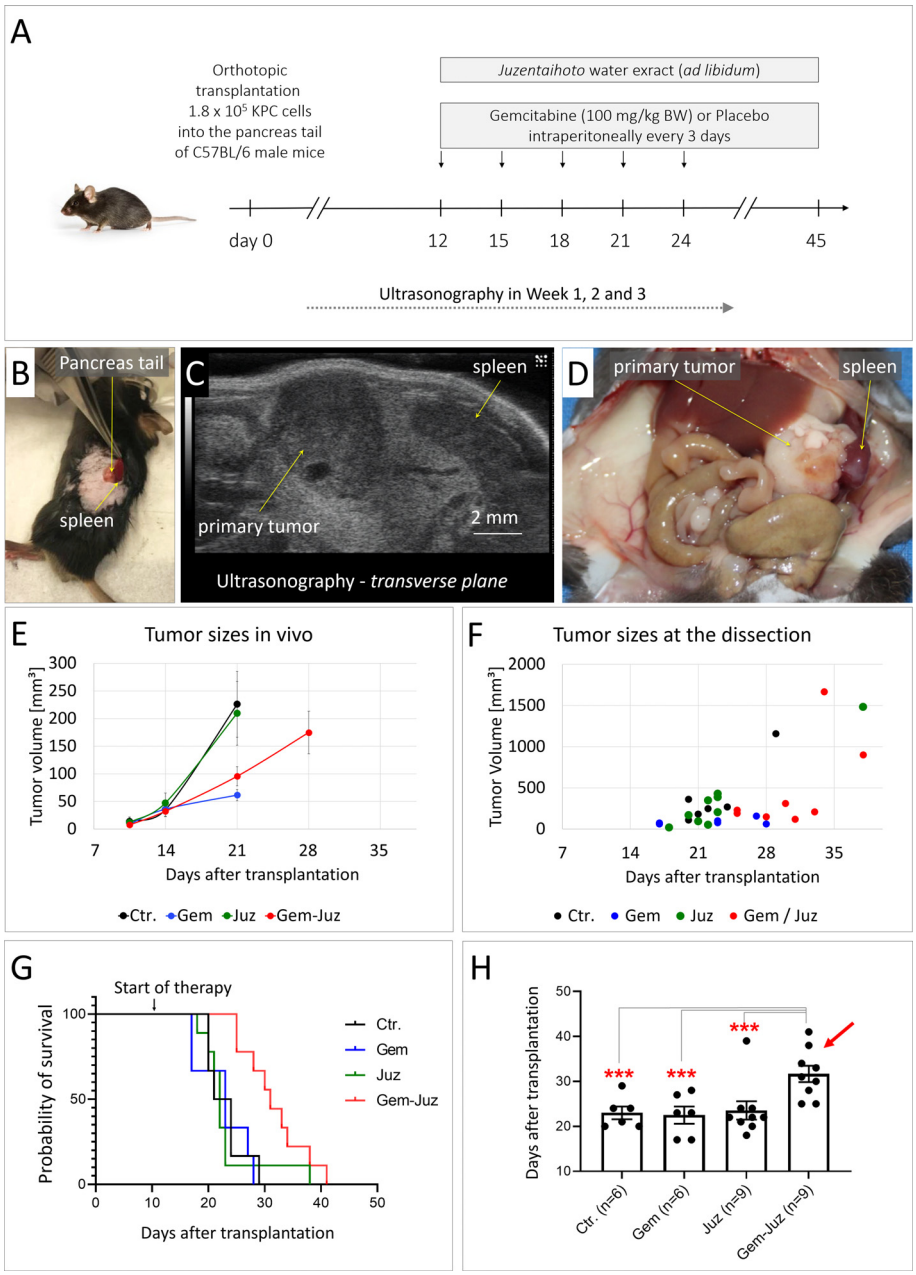

FIGURE 1
Effect of Gemcitabine and Juzentaihoto on tumor growth and overall survival. **(A)** Treatment scheme (controls received placebo = 0.9% NaCl) with **(B)** orthotopic syngenic transplantation of murine pancreatic cancer KPC cells into the pancreatic tale of C57BL/6 mice. **(C)** Example of ultrasound visualization of the primary tumor; here, the transversal plane is shown. **(D)** At autopsy: visualization of the primary tumor and spleen with metastasis. **(E)** Tumor growth monitored with ultrasonography over time. Error bars correspond to the SEM. Note, that the average tumor size at week 4 could only be calculated for the group treated with Gem-Juz, as the survival time was shorter in the other groups. **(F)** Tumor sizes at dissection. **(G)** Kaplan-Meyer analysis of survival within the different groups. **(H)** Average survival within the treatment groups, displayed as histogram, ***$p \leq 0.05$.

Hematoxylin-Eosin (H&E), Masson-Trichrom (MTS) and Berlin Blue staining, immunohistochemistry and immunofluorescence.

2.8 Histochemistry and immunohistochemistry

Note that all staining was performed on sequential tissue sections. For the representative histological images, tumors were selected from mice sacrificed at similar times for all treatment groups (approximately 3 weeks) after transplantation. This means that even for the groups with prolonged average survival, a mouse was selected that died approximately 3 weeks after transplantation. The selection of areas in the consecutive microscopic images aimed to include the tumor tissue, the invasion front, and if possible any remaining pancreatic tissue.

For all staining, 2.5 µm thick tissue sections (tumor with adjacent pancreas) were deparaffinized, rehydrated and pretreated

at 98°C for 20 min in citrate buffer (pH 6.0, Dako). Histochemical staining was performed on formalin-fixed and paraffin-embedded tissue sections as previously described (28).

For immunhistochemistry, endogenous peroxidase activity was inhibited on tissue sections with 3% H2O2 and unspecific binding sites were blocked with SEA BLOCK blocking buffer for 20 min. Slices were stained with primary antibodies against the following antigens: CD68 (Abcam, ab125212, 1:500), MHC II (1:100, Biolegend), CD163 (Abcam, ab182422, 1:500), Lipocalin (Abcam, ab 216462, 1:2,000), CD45 (Abcam, 208022, 1:1,000), CD3 (Abcam, ab5690, 1:100), CD4 (Abcam, ab237722, 1:1,000), CD8 (1:500, Abcam, ab228965), Ki-67 (Cell Marque, 275R-14, 1:200), and α-SMA (Dako, Clone 1A4, 1:250, RRID: AB_2335694), overnight at 4°C.

Finally, all slides were incubated with a corresponding HRP-labeled secondary antibody (Histofine; ready-to-use), stained with AEC (3-amino-9-ethylcarbazole) substrate (Pharmingen), washed with dH2O, counterstained with H&E, mounted with aqueous mounting medium (Aquatex), and dried overnight at RT. The images were acquired using the Axiovert 200M microscope (Leica) equipped with an AxioCamHR camera and processed with ImageJ (version 2.9.0/1.53t) (26).

2.9 Cell counting of immune cells in histological slides

In order to quantify macrophage infiltration, the stained slices were initially scored in a blinded fashion by two independent observers (score 1-5, with 1 = none to minor macrophage infiltration and 5 = high infiltration). For further quantification and validation of macrophage (CD68-positive) and leucocyte (CD45-positive) numbers, automated cell counting was performed using ImageJ (26). Briefly, the following areas were separately analyzed for each mouse: i) tumor center, ii) tumor border (invasion front), and iii) remaining pancreatic tissue. Four images were randomly recorded for each of these areas, resulting in a total of 12 analyzed areas per mouse. The analyzed regions measured 708 × 530 µm. Images were captured using an Axiovert 200M microscope with a 20× objective. For each staining, a macro was developed and visually inspected to ensure the accurate selection of various cell types, focusing on the identification of positively stained cells. Color deconvolution was used to generate a black-and-white picture, a threshold was defined, and noise was reduced. A watershed system allowed to distinguish neighboring cells, and cells were counted automatically using Image J. The median number of positive cells per area (μm^2) per mouse was calculated automatically with CSV-tables.

2.10 Molecular docking analysis

The 3D-structure of the interleukin 6 receptor (PDB ID: 7dc8) was retrieved from the RCSB Protein Data Bank (rcsb.org). Hetero-atoms and water molecules were deleted, polar hydrogen atoms were added, missing atoms were repaired, Kollman charges were added and finally saved in PDBQT format on AutoDockTools 1.5.6 (https://ccsb.scripps.edu/mgltools/). The prescreening was performed with PyRx AutoDock VINA (blind docking mode) to monitor docking to the entire surface of IL6R, and the Lamarckian algorithm of AutoDock VINA was chosen for the defined docking mode). The Lamarckian algorithm was used to analyze the docking poses and binding energies as described (29, 30). Three independent docking calculations were conducted with 25,000,000 energy evaluations and 250 runs by using the Lamarckian genetic algorithm. Visual Molecular Dynamics software was used for visualization (https://www.ks.uiuc.edu/Research/vmd/).

2.11 Statistical analysis

The statistical analysis including Kaplan-Meier survival analysis and graphic display were done with GraphPad Prism (9.5.0). The values for the cell counts are expressed as median ± SE (standard error).

Nested one-way ANOVA was conducted for the analysis, comparing the four groups with three areas for each group (tumor, tumor border, and pancreatic tissue). If only one specific tissue type was analyzed, an ordinary 1-way ANOVA was performed. For Image J, 5% of the outliers were excluded.

For experiments with three biological and three technical replicates (Western Blot and qRT-PCR), the mean value for the biological replicate includes the mean of the technical replicates.

P values ≤ 0.05 were considered statistically significant. P-values ≤ 0.05, ≤ 0.01, ≤ 0.001, and ≤ 0.0001 are denoted with *, **, ***, and ****, respectively. Non-significant results are indicated as 'ns'.

3 Results

The composition of the Juzentaihoto formulation was confirmed by HPLC. The chromatograms showed the specific peaks of Juzentaihoto with visualization of the significant lead compounds via the retention time and the UV7VIS spectra (Supplementary Figure S1) (22).

3.1 Survival is independent of tumor growth

To investigate the effect of Juzentaihoto *in vivo*, the aqueous extract of Juzentaihoto was administered *ad libitum* to mice with orthotopic PDAC tumors, either alone or in combination with Gemcitabine. Gemcitabine was applied intraperitoneally at a dose of 100 mg/kg BW every three days.

At the time of tumor cell transplantation, the average animal weight was 25.1 ± 1.5g. At day 8/9 after orthotopic transplantation of KPC cells, when the average tumor size measured by ultrasonography (US) was 10.4 +/- 18.4 mm^3, mice were divided into 4 treatment groups; group 1: control (n = 6), group 2: Gemcitabine (n = 6), group 3: Juzentaihoto (n = 9) and group 4: Gemcitabine and Juzentaihoto (n = 9). The average tumor size in the control group was 13.7 ± 20.9 mm^3, in the Gemcitabine group

9.4 ± 13 mm³, in the *Juzentaihoto* group 11.8 ± 27.8 mm³ and in the Gemcitabine and *Juzentaihoto* group 7.7 ± 7.9 mm³. The average animal weight was: 25.4 ± 0.8 g in the control, 25.6 ± 1.8 g in Gemcitabine, 24.9 ± 1.6 g in *Juzentaihoto* and 25.2 ± 1.8 g Gemcitabine and *Juzentaihoto* groups. All treatments started at day 12 (Figure 1A). All treatments started at day 12 (Figure 1A).

In the Juzentaihoto-treated groups, the water was replaced by the Juzentaihoto decoction, which was well-tolerated and drunk ad libitum. On average, a mouse drinks around 15 ml of fluid per 100 grams of its body weight daily, which corresponds to approximately 3.75 ml of fluid for a 25 g mouse. This amounts to about 150 g/kg body weight per mouse. There were no symptoms of dehydration, diarrhea or weight loss that could be attributed to Juzentaihoto treatment. Nevertheless, and as expected during disease progression, mouse weight decreased individually with time-to-death. However, no significant difference in body weights was observed within the different groups with high inter-mouse variability (Supplementary Figure S2).

Juzentaihoto alone had no effect on tumor growth, with average tumor sizes almost identical within the control- and the Juzentaihoto-groups. As shown in the Figure 1E, at week 3 (~ at the time of the 4th Gemcitabine dose), the average tumor size measured with US in control- and Juzentaihoto- treatment groups were 225.9 ± 103.5 and 209.7 ± 115.5 mm³, respectively. Gemcitabine chemotherapy on the other hand, led to a significantly decelerated tumor growth, whether used alone (61.6 ± 20.1 mm³) or in combination with Juzentaihoto (95.5 ± 52.2 mm³). This is even more prominent at the later stages of the treatment, as shown in the Figure 1F, where tumor sizes at the time of dissection for each individual animal are presented.

The decrease in primary tumor growth however did not translate into survival (Figures 1G, H). At the time of autopsy, metastases had remained local (*i.e.*, infiltration into the spleen). Distant metastases into the liver were not observed. While Gemcitabine monotherapy effectively reduced tumor growth, it failed to prolong mice survival. Mean overall survival in the control group was 23.0 days (± 3.5) and in the groups treated with Gemcitabine or Juzentaihoto monotherapy 22.5 days (± 4.7) and 23.6 days (± 6.1), respectively. Only supportive treatment with Juzentaihoto extract together with Gemcitabine increased life expectancy significantly: Mice in the combination treatment arm showed an average survival of 31.7 days (± 5.5), which was ~ 38% longer than the average survival of controls.

3.2 Histochemical analysis of tumor response

In H&E staining all treatment groups showed infiltration by tumor cells into the pancreatic tissue with separation of single acini from the lobe structure and an inflammatory infiltrate (Figure 2A shows representative images, from about 3 weeks after transplantation). With combination-treatment (Gem/Juz) the tumor itself appeared looser with surrounding acini more contiguous than in Gemcitabine treatment alone.

While pancreatic cancer in patients is usually characterized by a dense fibroblastic tumor stroma, the mesenchymal tumor stroma in our orthotopic KPC transplantation model appears to be negligible and not significantly differently between the four groups: the Masson Trichrom staining (MTS) revealed only a minor collagen deposition (Figure 2B). Also, only a moderate expression of alpha-smooth muscle actin (α-SMA) was observed within the tumor tissue, probably in myofibroblasts and small tumor vessels. (Figure 2C).

All tumors were highly proliferative, as confirmed by Ki-67 staining (which associates cell-cycle- dependent with various types of chromatin) (Figure 2D). Ki-67$^+$ cells were preferentially located at the tumor periphery in all four groups. However, Ki-67$^+$ cells appeared to be more prominent in tumors of mice treated with Gemcitabine as compared to the combination treatment (Gem/Juz).

3.3 Immunohistochemical analysis of the immune-cell microenvironment

Immune microenvironment and immune cell infiltration of tumors is often closely related to clinical outcomes. To determine whether immune-cell infiltration in our KPC mouse model was influenced by the various treatments, sequential slides were stained with a macrophage detecting anti-CD68 antibody. Whilst Juzentaihoto alone did not significantly alter CD68$^+$ macrophage infiltration, we observed an increase upon Gemcitabine treatment, especially at the tumor border, which was even higher in the Gem/Juz combination treatment (Figure 3A). In order to quantify macrophage infiltration within the tumor tissue, the stained slices were scored in a blinded fashion by two independent observers (score 1-5, with 1 = none to minor infiltration and 5 = high infiltration). As shown in Figure 3B, the scoring of CD68+ macrophages confirmed our qualitative observations and showed significantly higher scoring in the combination treatment when compared to control ($p = 0.0002$) and Juzentaihoto ($p < 0.0001$) groups. Increased macrophage infiltration was also observed after Gemcitabine monotherapy (in comparison to controls ($p = 0.03$)), with a clear, although not significant ($p = 0.23$) further increase after Gem/Juz treatment. CD68$^+$ cells were further quantified using Image J (Figures 3C–E). Here, three areas were selected for quantification: tumor tissue, adjacent pancreatic tissue and the tumor margin at the transition to pancreatic tissue (invasion front). As depicted in Figures 3C–E, the digitized analysis corroborated our initial visual assessment, revealing a notable increase in CD68$^+$ macrophages following combination treatment, particularly at the tumor border. Additionally, there was a discernible, although - at the border not statistically significant - increase observed with Gemcitabine monotherapy when compared to the control group.

As CD68 serves as a general macrophage marker, we proceeded to differentiate M2 macrophages. Thus, we performed IHC for MHC Class II and CD163. Visual evaluation of MHCII staining showed negligible numbers of MHCII$^+$ cells without apparent differences within the four groups (Figure 3F). Similarly, CD163$^+$ M2

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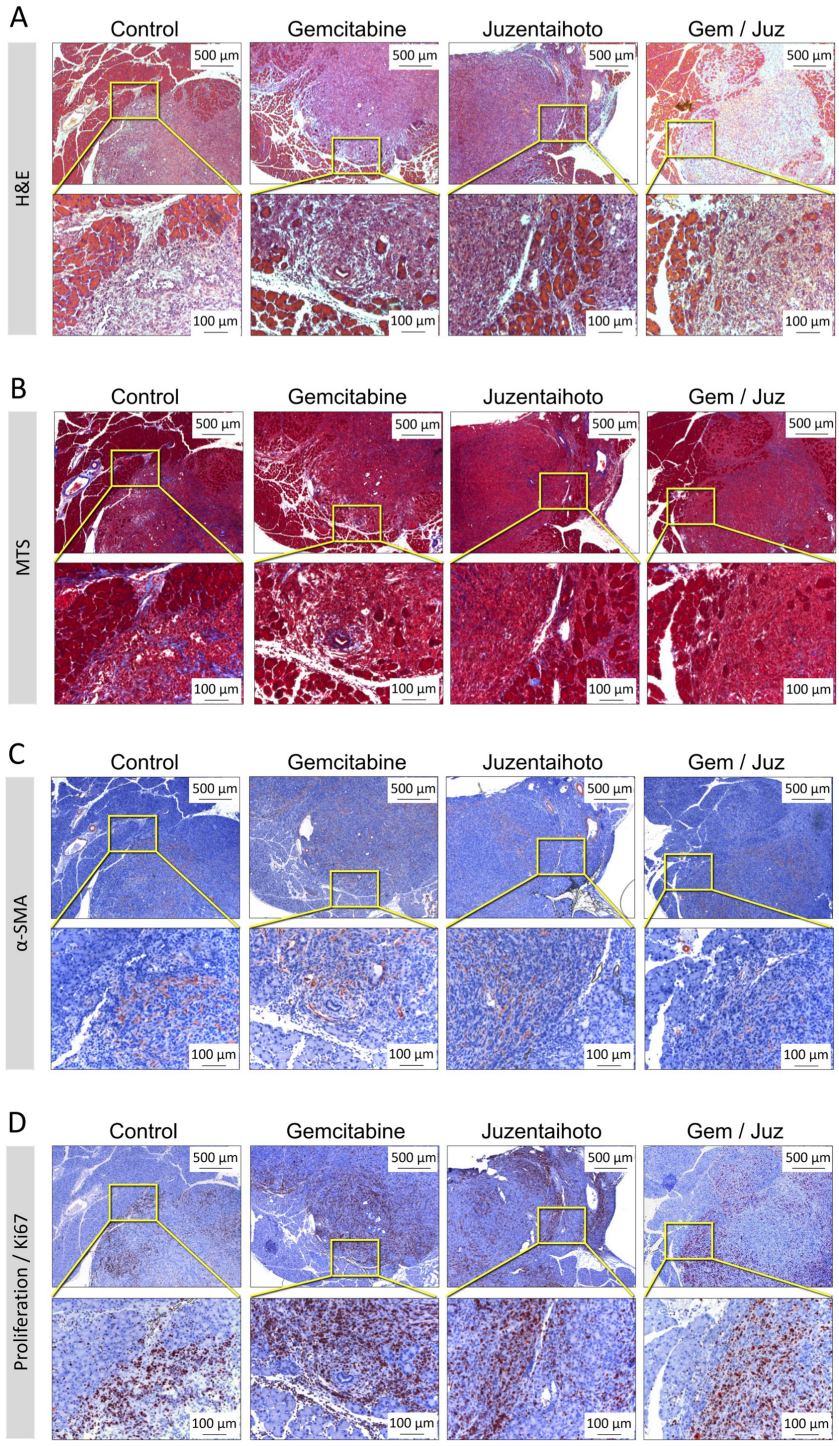

FIGURE 2
Representative microscopic images of the general tumor morphology among treatment groups performed on serial sections. **(A)** H&E staining, **(B)** Masson Trichrome staining (MTS), **(C)** IHC of α-smooth muscle actin (α-SMA) and **(D)** Ki-67 staining. Images show tumor slices taken from representative mice approximately 3 weeks after transplantation. Scale bars correspond to 500 µm (upper row) and 100 µm (lower row), respectively.

macrophages were also found to be infrequent (Figure 3G). These findings suggest that the increased number of macrophages, especially at the tumor border, observed upon Gemcitabine and combination treatment predominantly consisted of M1 macrophages.

Besides being a marker for M2-macrophages, CD163 is known as high affinity scavenger receptor for the hemoglobin-haptoglobin complex (31). Scavenging of iron within the complex protects against its proinflammatory and potentially toxic effects. It is, thus, interesting to note that in our study, CD163 apparently stains heme complexes within tumor-adjacent acinar cells, rather than depicting individual immune cells (Figure 3G; arrows). This was confirmed when we stained for lipocalin-2, an iron sequestering

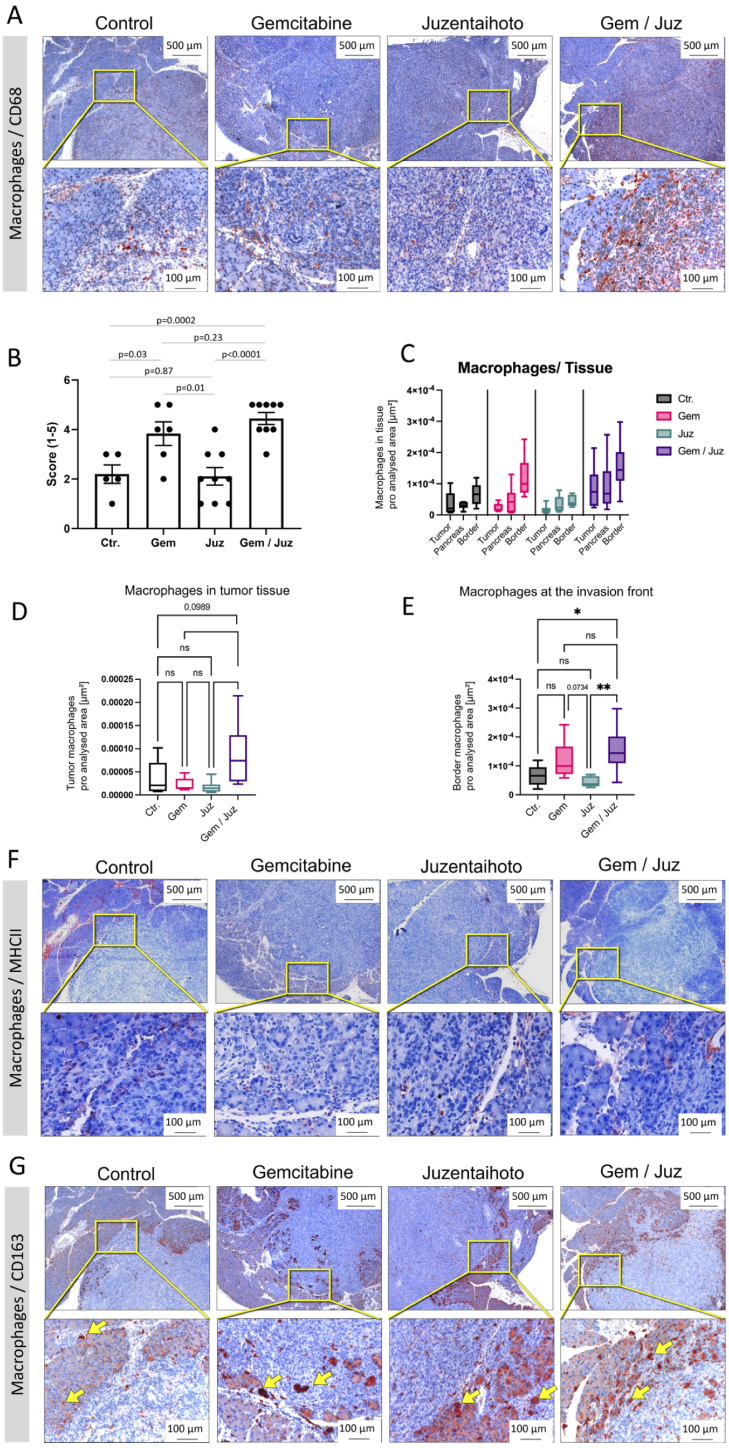

FIGURE 3
Macrophage infiltration of KPC tumors upon treatment of mice with Gemcitabine, Juzentaihoto, and their combination. Staining was performed on sequential slices. **(A)** CD68 staining of representative sequential tumor sections of the four treatment groups. Note the significant increase in $CD68^+$ cells after combination treatment (Gem/Juz). **(B)** Scoring of $CD68^+$ macrophages within the four groups (n = 6 for placebo and Gemcitabine and n = 9 for Juzentaihoto and Gem/Juz groups). **(C-E)** Image J-based quantifications and comparisons of the macrophage infiltration within the primary tumor, at the tumor border (infiltration front) and within the adjacent pancreatic tissue. Results confirm the significant increase of $CD68^+$ macrophages after combination treatment (Gem/Juz), especially at the tumor border. **(F)** MHCII staining at the tumor infiltration front. Only scarce $MHCII^+$ cells are visible, even at higher magnification (40x). **(G)** CD163 staining at the tumor infiltration front. Rather than depicting macrophages, the antibody stains the hemoglobin-haptoglobin complex of tumor-adjacent acinar cells (yellow arrows, see below). Scale bars correspond to 500 µm (upper row) and 100 µm (lower row), respectively. P-values ≤ 0.05 and ≤ 0.01 are denoted by * and **, respectively. Non-significant results are indicated as 'ns'. P-values close to a significance level are displayed as numbers.

protein (Supplementary Figure S3A). Gemcitabine especially seemed to trigger iron uptake, whilst free iron deposits (Berlin blue reaction) were scarce (Supplementary Figure S3B).

Staining for T-cells (CD3, CD45) as well as their subgroups: cytotoxic T-cells (CD8) and T-helper cells (CD4) did not show significant overall-changes throughout the tissue. The numbers of $CD8^+$ and $CD4^+$ cells were too minor for a meaningful sub-analysis. Supplementary Figure S4 exemplarily shows CD45 immunostaining. For $CD45^+$ lymphocytes at the tumor border, Image J-supported subgroup-analysis revealed a significant increase after Gemcitabine treatment (compared to controls).

Whilst Gemcitabine treatment inhibits tumor growth and affects lymphocyte numbers at the tumor border, its combination with Juzentaihoto seems to affect macrophage numbers, especially at the tumor front.

3.4 Behavior of KPC cells *in vitro* in response to Juzentaihoto and combination treatment

To assess cellular viability *in vitro*, KPC cells were treated for 72 h with increasing concentrations of Gemcitabine (0-200 nM), Juzentaihoto (1:10 – 1:1000), a combination of the two, or solvent alone as control. Cell growth was monitored over time using the Incucyte live cell imaging system, and the IC_{50} was calculated based on cell confluency after 72 h treatment (Supplementary Figures S5A, B). As in the mouse experiments, Juzentaihoto alone did not inhibit tumor cell growth. Gemcitabine and the combination of Gem/Juz however showed a strong and significant growth inhibitory effect on KPC tumor cells attributable to the cytotoxic effect of Gemcitabine, with IC_{50} of 13.5 nM and 21.6 nM, respectively.

With the aim of analyzing cellular differentiation in view of epithelial-to-mesenchymal transition within the various treatment groups and control, double fluorescent staining was performed using an antibody against E-cadherin for epithelial cells and vimentin, respectively for stromal/mesenchymal cells. As shown in the Supplementary Figure S5C, the majority of control KPC cells exhibited an epithelial phenotype, as evidenced by positive staining for E-cadherin, with fewer vimentin-positive mesenchymal cells. A similar morphology/phenotype was observed in KPC cells after 24 h Juzentaihoto treatment (1:20). Despite a significantly reduced cell viability observed in both Gemcitabine-treated (80 nM) and double-treated cells due to treatment, the surviving cells maintained their differentiation (with E-cadherin positivity) at this time (Supplementary Figure S5C).

3.5 Chemokine expression by tumor cells *in vitro*

To better understand the mechanisms responsible for macrophage infiltration and immune modulatory mechanisms, we analyzed chemokine expression by tumor cells *in vitro*. Consequently, a chemokine-cytokine array was performed after 24 h incubation of KPC cells with control, Gemcitabine (80 nM), Juzentaihoto (1:20), or both.

Juzentaihoto treatment as well as the combination treatment (but not Gemcitabine treatment alone) showed a significant increase of the macrophage chemoattractant chemokines CCL2/MCP-1, CCL20/MIP3α, and CXCL2/MIP-2 in the supernatant of tumor cells (Figures 4A–C; Supplementary Figure S6). Furthermore, granulocyte macrophage colony stimulating factor (GM-CSF) was also increased by Juzentaihoto treatment and combination treatment, however to a lower extend (Figure 4D). These results suggest that following Juzentaihoto treatment, tumor cells may release chemoattractive cytokines, indicating a potential correlation with the observed increase in macrophage infiltration. The treatment with Juzentaihoto appeared to stimulate the secretion of signaling molecules by tumor cells, possibly contributing to the enhanced recruitment of macrophages to the tumor microenvironment.

To study, whether Juzentaihoto can also directly affect macrophages causing their activation, we performed cytokine expression analyses, incubating macrophages of the murine macrophage MH-S cell line with the different treatments (control, Gemcitabine, Juzentaihoto, or their combination).

3.6 Chemokine/cytokine expression by macrophages *in vitro*

As shown in Figures 5A, B (see also Supplementary Figure S6), the classical pro-inflammatory M1-cytokines IL-6 and TNF-α were increased upon macrophage-stimulation with Juzentaihoto or combination treatment (but not Gemcitabine alone). This strongly suggests, that Juzentaihoto treatment not only induces KPC tumor cells to produce macrophage attracting chemokines (Figure 4), but also acts on macrophages, reprogramming them to the M1 phenotype. Similar to the inflammatory stage of wound healing, we further showed an induction of CXCL2/Gro2/MIP2 and to a lesser extent CXCL1/Gro1 as well as CCL20/MIP3α by Juzentaihoto. More than CXCL1/Gro1, which also has pro-angiogenic properties, the pro-angiogenic factor VEGF itself was increased upon Juzentaihoto treatment (Figures 5C–F). Furthermore, IL-12 – a T-cell costimulatory factor (32) was induced – (Figure 5G). At the same time, cytokines which are involved in the prevention of overshooting immune reactions were also elevated by Juzentaihoto, such as Interleukin 1 response element (IL-1ra) which abrogates NF-κB activation, and the γ-interferon response element CXCL10/IP10 which is involved in anti-tumor activity (Figures 5H, I).

3.7 Molecular docking analysis

The 3D-structure of the interleukin 6 receptor (PDB ID: 7dc8) was retrieved from the RCSB Protein Data Bank (rcsb.org) to monitor docking to the entire surface of IL6R. Virtual comparison with an online databank showed that albiflorin, benzoylpaeoniflorin, and glycyrrhizin interact with the IL6 receptor.

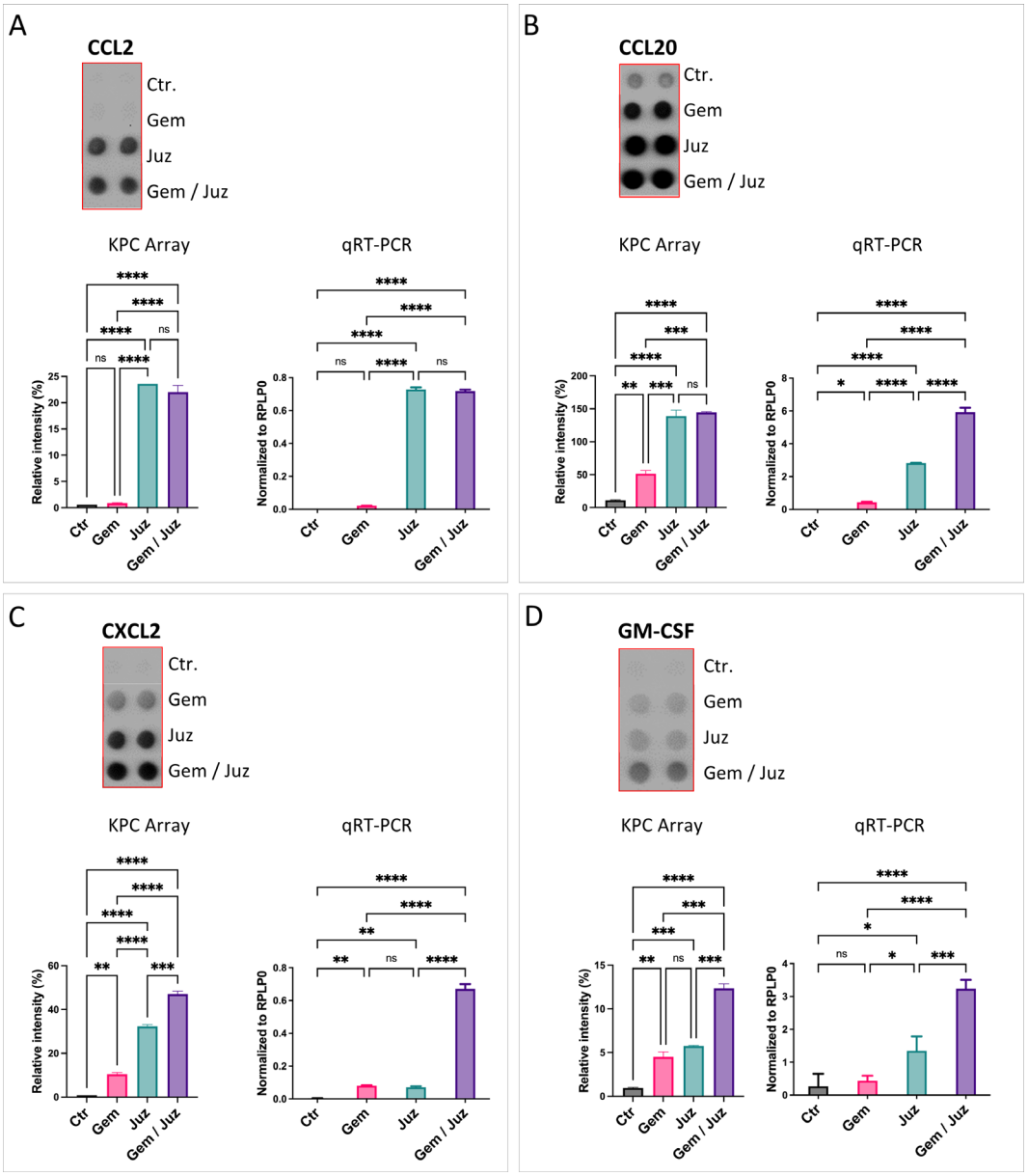

FIGURE 4
Expression and secretion of macrophage chemoattractant cytokines **(A)** CCL2, **(B)** CCL20, **(C)** CXCL2, and **(D)** GM-CSF by KPC cells upon treatment with Gemcitabine (80nM), Juzentaihoto (1:20 in medium with 1% DMSO), and their combination. Chemokine Arrays are shown as dot-blots (refer also to Supplementary Figure S6) and corresponding histograms after quantification. The expression of cytokines (CCL2, CCL20, CXCL2, and GM-CSF) was verified at the RNA level with qRT-PCR. Notably, Juzentaihoto treatment and combination therapy (but not Gemcitabine treatment alone) demonstrated a significant increase in the expression of macrophage chemoattractive/stimulating cytokines: **(A)** CCL2, **(B)** CCL20, **(C)** CXCL2, and **(D)** GM-CSF. P-values ≤ 0.05, ≤ 0.01, ≤ 0.001, and ≤ 0.0001 are denoted with *, **, ***, and ****, respectively. Non-significant results are indicated as 'ns'.

4 Discussion

Tumor-stroma interaction influences tumor progression and its response to chemotherapy (33). The idea of a tumor as 'wound that does not heal' still holds.

Herbal medicine has always been employed to balance immune reactions as well as redox-mechanisms and ameliorate microcirculation and nutrition (6). The ancient prescription Juzentaihoto which has been used to treat cachexia for centuries, has - in recent years - played an increasing role in the supportive treatment of cancer patients, especially those with pancreatic cancer (6, 11–16). It is therefore intriguing to explore the effect of Juzentaihoto on survival and tumor microenvironment in the immune-competent KPC mouse transplantation model of pancreatic cancer, especially in the context of the chemotherapeutic agent Gemcitabine. KPC cells carry both, a mutation in the oncogene KRAS (LSL-KrasG12D/+) and in the tumor-suppressor gene P53 (LSL-Trp53R172H/+), which makes the tumor in our experimental model rather aggressive.

We showed that combination treatment of Gemcitabine and Juzentaihoto significantly prolonged survival of KPC tumor bearing mice not only, when compared to placebo-treated mice (+ 8.67

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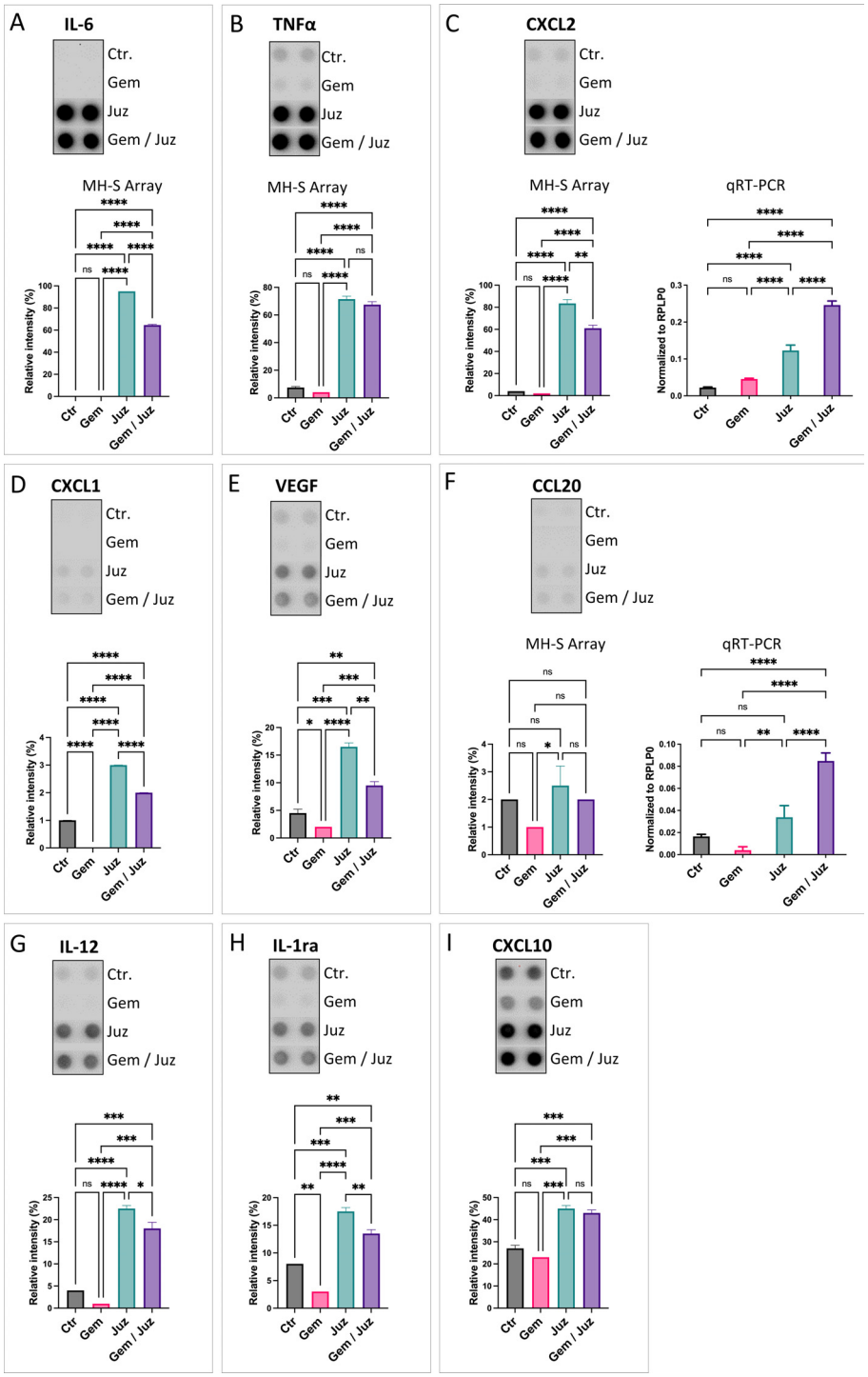

FIGURE 5
Chemokine secretion upon treatment of MH-S macrophages with Gemcitabine (80 nM), Juzentaihoto (1:20 in medium with 1% DMSO), and the combination of both. **(A)** IL-6, **(B)** TNF-α, **(C)** CXCL2/MIP2, **(D)** CXCL1/Gro1, **(E)** VEGF, **(F)** CCL20/MIP3, **(G)** IL-12, **(H)** IL-1ra, and **(I)** CXCL10. Chemokine arrays are shown as dot-blots and corresponding histograms after quantification. For C) and F) the protein expression was verified at the RNA level with qRT-PCR. P-values ≤ 0.05, ≤ 0.01, ≤ 0.001, and ≤ 0.0001 are denoted with *, **, ***, and ****, respectively. Non-significant results are indicated as 'ns'.

days; 37.7%), but also compared to mice treated with Juzentaihoto (+ 8.11 days) or Gemcitabine alone (+ 9.17 days). In cell culture, Gemcitabine (as chemotherapeutic agent) showed (alone or in combination with Juzentaihoto) the expected cell depletory effect on KPC tumor cells. Similarly, *in vivo* tumor growth was decreased in Gemcitabine-treated mice, as well as with combination treatment. The effect of Gemcitabine on tumor volume did not, however, translate into prolonged survival. We can conclude that

the trigger of the combination treatment for a better survival rate must be located elsewhere, and it is self-suggestive to look into the tumor microenvironment.

Tumors shape their own microenvironment. The immune system responds to the tumor in the context of its microenvironment which varies according to tumor stage. To some extent, the genetic background of the tumor contributes (34). In this context, we formerly showed that expression of tumor-related oncogenes and loss of tumor-suppressor genes influence chemokine expression (35).

Pancreatic adenocarcinomas are generally considered non-immunogenic, displaying low infiltrates of cytotoxic T-lymphocytes (CTLs) along the invasive margin but not within the tumor core (36). This is in accordance with our observations, where T-cells ($CD45^+$) were visible at the infiltration front and increased after Gemcitabine treatment – with the unmasking of tumor-epitopes. Recognition and killing of transformed cells by effector T-cells (NK and cytotoxic $CD8^+$ cells) takes place in the early elimination phase, when the tumor is not-yet host to its own microenvironment (37). Accordingly, the numbers of $CD8^+$ and $CD4^+$ cells in our experiments were negligible.

Whilst the tumor modifies the microenvironment to limit the host response, the adaptive immune system reacts to tumor specific antigens. Tumor-associated macrophages (TAMs) can be of an activated M1 or an immune-suppressive M2 phenotype. Whilst the M1 phenotype secretes cytokines such as IL6, TNF-α, and CCL2 and is involved in the synthesis of reactive oxygen-species (ROS), the M2 phenotype seems to be better adapted at scavenging debris, promoting angiogenesis and tissue remodeling (38–40). This phenotype has thus been termed pro-tumorigenic. Halbrook, et al. showed that tumor-associated (M2) macrophages (TAM) release a spectrum of pyrimidine species. These include deoxycytidine. Gemcitabine differs from deoxycytidine only by two Fluorine atoms at the deoxyribose of the nucleoside cytidine (Supplementary Figure S7). Due to the similarity in molecular structure, Gemcitabine and deoxycytidine compete intracellularly for deoxycytidine kinase, the enzyme necessary for Gemcitabine activation. Halbrook, et al. showed that deoxycytidine blocks the cytotoxic effect of Gemcitabine dose-dependently. As a consequence, in an M2 microenvironment, Gemcitabine activation and activity as a chemotherapeutic drug decreases (41). Thus, tumor-associated (M2) macrophages not only scavenge debris and promote tumor growth, they also inhibit Gemcitabine activity (38–41). Whilst it is known that M2 macrophages are associated with poor prognosis (36, 42), they are also involved in tumor cell invasion (43). Macrophage activation to the anti-tumor M1 phenotype can explain the overall better prognosis in the combined treatment group with ameliorated efficacy of Gemcitabine.

It is known that tumor epitopes can be unmasked by chemotherapy which further leads to better macrophage recognition. Such antigens are derived from proteins involved in the DNA-damage response (44, 45). Accordingly, amino acid anchor-residue modifications and changes in peptide length render peptides to favor surface expression of alternative HLA-alleles with increased immunogenicity (46). As Gemcitabine unmasks epitopes important for macrophage recognition, it is intriguing to ask whether Gemcitabine would be more effective in an inflammatory background.

The presentation of exogenous antigens on MHC class I molecules is vital for the detection of cancer by immune cells (46). Our results are coherent with reports on histopathological

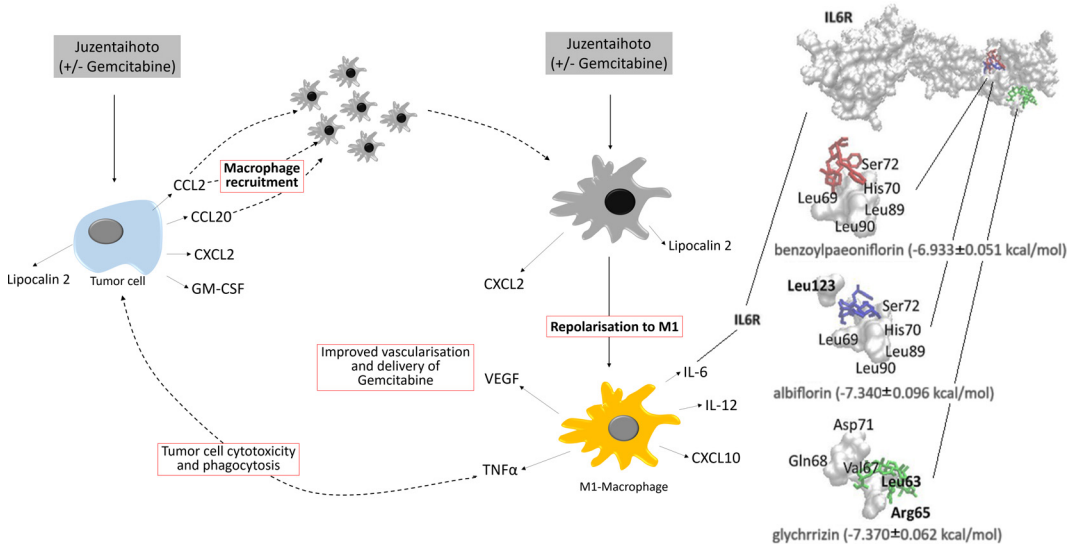

FIGURE 6
Postulated effect of Juzentaihoto in combination with Gemcitabine in a KPC model of pancreatic cancer. Left: Upon treatment with Juzentaihoto alone or in combination with Gemcitabine, but not Gemcitabine alone, pancreatic cancer cells release macrophage-chemoattractive cytokines (CCL2, CCL20, CXCL2) as well as macrophage colony stimulating factor (GM-CSF). Right: Upon treatment with Juzentaihoto (alone or in combination with Gemcitabine), macrophages are repolarized to the IL-6, IL-12, TNF-α and CXCL10-producing M1 macrophages known to have anti-tumoral properties. Note that IL-6 is increased upon Juzentaihoto-treatment. Using a virtual interaction map (far right) we showed that acting constituents of Juzentaihoto bound to the IL-6 receptor.

findings from patients with pancreatic cancer, where a high density of macrophages at the tumor border was associated with an ameliorated response to chemotherapy (42). The authors propose that the number of macrophages should be taken into account when selecting patients for chemotherapy with Gemcitabine (42).

In our tumor mouse model, we observed a significant increase in $CD68^+$ macrophages in response to combination treatment with Gemcitabine and Juzentaihoto. Accordingly, we showed in pancreatic cancer cells (KPC) *in vitro* an induction of monocyte/macrophage-chemoattractant cytokines such as CCL2/MCP1, CCL20/MIP3α, CXCL2/MIP2α and also GM-CSF by Juzentaihoto (and the combination). These results suggest that combination treatment induces macrophage chemotaxis and activation, ultimately leading to the induction of anti-tumor immunity (47).

Furthermore, Juzentaihoto (and its combination with Gemcitabine, but not Gemcitabine alone) increased the production and release of acute phase cytokines (IL-6, TNF-α) from macrophages, as well as of pro-inflammatory chemokines such as CXCL2/MIP2α/Gro2, and to a smaller extend CXCL1/Gro1 and CCL20/MIP3α. Juzentaihoto also increased interleukin receptor antagonist IL-1ra and CXCL10/IP10, thus suggesting the modulation of IL-1 and interferon-related immune reactions, preventing overshooting immune reactions.

In silico research allows to predict interaction of the acting constituents of Juzentaihoto with various protein residues (Figure 6). Virtual comparison with an online databank showed that albiflorin, benzoylpaeoniflorin, and glycyrrhizin interact with the IL6 receptor. Like Gemcitabine, Juzentaihoto has various acting modalities. Whilst Juzentaihoto induces macrophages to produce cytokines like IL6 and Tumor necrosis factor (TNF- α) in our experiments, some of its active constituents (from ginseng and glycyrrhizae *radix*) hinder the inflammatory arm of IL6 signaling, thus preventing muscle wasting and cachexia (6). It has further been shown that Isoliquiritigenin, a flavonoid compound of Glycyrrhizae root blocks M2 macrophage-polarization (48). It is one column of the adaptogenic effect against cachexia (6), which shall be explored further.

Another double-edged sword is the amelioration of microcirculation. Whilst common strategies try to prevent neoangiogenesis in tumors by inhibition of VEGF, wound healing involves the amelioration of microcirculation. Also because of its fibrosis, pancreatic cancer is rather resistant to chemo- and radiotherapy. VEGF-inhibitors are thus not employed. However, it would be reasonable to ameliorate microcirculation in this context. We showed that Juzentaihoto enhances VEGF expression by macrophages. On the other hand, it has been shown that Juzentaihoto suppresses tumor-induced angiogenesis in B16 melanoma cells *in vivo* and *in vitro* (49). It follows that the significance of Juzentaihoto as pro- or antiangiogenic factor varies in view of the cellular background, *i.e.*, immune- or tumor cell. Further research would be necessary in this regard.

From our study we conclude that Juzentaihoto-induced polarization of tumor-associated macrophages into the M1 phenotype not only induces anti-tumor immune-cell activity and cytokine release (such as TNF-α, IL6), it also ameliorates Gemcitabine efficacy in view of DNA-analogue as well as partial antitumor antigen.

Taken together, our results suggest that the combination treatment of Gemcitabine and Juzentaihoto changes the microenvironment of pancreatic cancer and thus prolongs survival. Our results support Japanese studies suggesting that Kampo medicine, especially Juzentaihoto, can be a supplementary treatment option, especially in pancreatic cancer.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Ethics statement

The animal study was approved by LAVES, Niedersächsisches Landesamt für Verbraucherschutz und Lebensmittelsicherheit (33.9-42502-04-18/2953). The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

JN: Data curation, Formal analysis, Investigation, Methodology, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. PS: Data curation, Formal analysis, Investigation, Software, Visualization, Writing – original draft, Writing – review & editing. HR: Data curation, Formal analysis, Software, Writing – original draft, Writing – review & editing, Conceptualization, Funding acquisition, Methodology, Resources, Validation. KK: Conceptualization, Methodology, Validation, Writing – original draft, Writing – review & editing. TE: Writing – original draft, Writing – review & editing. FA: Writing – original draft, Writing – review & editing, Investigation, Project administration, Supervision, Validation, Visualization. VE: Supervision, Validation, Writing – original draft, Writing – review & editing, Conceptualization, Funding acquisition. SC: Conceptualization, Funding acquisition, Supervision, Validation, Writing – original draft, Writing – review & editing, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Visualization.

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Conflict of interest

Author HR was employed by company Phytochem Reference Substances.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

TE and KK declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2024.1454291/full#supplementary-material

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Case report: Achieving significant tumor reduction in advanced pancreatic adenocarcinoma

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Pancreatic cancer remains a highly malignant and challenging tumor with a dismal 5-year survival rate of only 13%. The majority of patients are diagnosed at advanced stages, where surgical options are limited, and prognosis is poor. Immunotherapy, particularly PD-1 inhibitors, has shown limited success in pancreatic cancer due to its unique tumor immune microenvironment. However, certain genetic profiles, such as BRCA1/2 mutations, high tumor mutational burden (TMB), or microsatellite instability-high (MSI-H), may enhance sensitivity to these therapies. This report presents two cases of advanced pancreatic cancer with BRCA1/2 mutations treated with a combination of chemotherapy and immune checkpoint inhibitors. The first patient, with TMB-H and stable microsatellites, achieved complete remission after conversion therapy and remains disease-free for over two years post-surgery. The second patient, with MSI-H and low TMB, experienced significant tumor regression and improved quality of life with a prolonged progression-free survival, although the patient ultimately declined surgery. These cases suggest that combined chemotherapy and immunotherapy may offer a promising treatment option for select pancreatic cancer patients, particularly those with specific genetic profiles, warranting further investigation into personalized approaches to immunotherapy in this malignancy.

KEYWORDS

pancreatic adenocarcinoma, tumor reduction, case report, immunotherapy, chemotherapy

Introduction

Pancreatic cancer is a highly malignant tumor of the digestive system with a 5-year survival rate of only 13% (1). It often lacks specific symptoms in the early stages, grows rapidly, and is prone to metastasis and recurrence. Most patients are diagnosed at an advanced stage with low surgical resection rates and poor prognosis. Previously, the special

tumor immune microenvironment of pancreatic cancer was thought to result in low drug sensitivity (2). As a result, PD-1 and other immune checkpoint inhibitors have not been broadly successful in treating pancreatic cancer (3, 4). However, emerging evidence suggests that certain genetic profiles, such as BRCA1/2 mutations, high tumor mutational burden (TMB-H), or microsatellite instability-high (MSI-H), may confer increased sensitivity to these therapies. This report presents two cases of advanced pancreatic cancer with BRCA1/2 mutations, who were treated with a combination of chemotherapy and immune checkpoint inhibitors. The outcomes suggest that such combined therapy may offer a viable treatment option for select patients with advanced disease. This manuscript follows the CARE guidelines for case reports to ensure the accuracy and transparency of the presented data.

Case presentation

Case 1

A 33-year-old male presented with a two-month history of abdominal pain, which had worsened over the past 20 days, accompanied by jaundice and melena. He had no significant past medical history and no relevant family history. Enhanced abdominal computed tomography (CT) revealed a slightly hypodense mass in the right lobe of the liver, measuring approximately 6.9 cm x 6.1 cm, with blurred edges, faint lobular changes, heterogeneous density, and marked ring enhancement during the arterial phase. Additionally, a homogeneously hypodense soft tissue mass was identified in the head of the pancreas, measuring about 5.4 cm x 3.4 cm, with ring enhancement (Supplementary Figure S1A). This pancreatic mass raised the suspicion of metastasis or primary pancreatic cancer. Laboratory tests showed the following results: alpha-fetoprotein (AFP) at 8.48 ng/ml, carcinoembryonic antigen (CEA) at 176 ng/ml, and serum carbohydrate antigen 19-9 (CA 19-9) exceeding 1000 U/ml. Based on imaging and tumor marker findings, a preliminary diagnosis of a malignant tumor in the pancreatic head with liver metastasis was made. The TNM staging was determined to be T2N1M1, Stage IV. Given the advanced nature of the pancreatic cancer, the patient was not a candidate for surgical intervention.

A laparoscopic biopsy of the liver and pancreas was performed. Postoperative pathology revealed poorly differentiated adenocarcinoma. Immunohistochemical staining of the liver biopsy was positive for CK7, CK8/18, CK19, and negative for Syn, CgA, P63, CK5/6, Heppar-1, GPC3, GS (+), TTF-1, CDX-2, SATB-2, DPC4 (±), Ki-67 (+, 80%), supporting the diagnosis of adenocarcinoma (Figure 1). Given the clinical presentation, the findings were consistent with liver metastasis from pancreatic cancer. Genetic analysis revealed the following: Microsatellite Stable (MSS) status and a Tumor Mutational Burden (TMB) of 9.84 mutations/Mb (high). Somatic mutations were identified in genes including TARX, BRCA1, BRCA2, BRIP1, CHD1, EPHA7, ERCC3, ETV1, FANCD2, INPP48, LRP18, MSH3, PIK3C2G, PTEN, RASA1, SMAD2, STAG2, STAT58, TAP2, and WRN.

The patient then received three cycles of neoadjuvant chemotherapy combined with immunotherapy over three months: Gemcitabine 1000 mg/m^2 on days 1 and 8, Cisplatin 25 mg/m^2 on days 1 and 8, and Camrelizumab 200 mg on day 1, every three weeks (Q3W). Following this treatment regimen, an enhanced abdominal CT scan revealed a slightly hypodense nodule in the right lobe of the liver, measuring approximately 2.3 x 2.8 cm with inhomogeneous density and unclear boundaries, displaying uneven enhancement (Supplementary Figure S1B). Additionally, a hypoechoic mass in the head of the pancreas, measuring about 2.0 x 2.2 cm, showed significant reduction in size compared to previous scans. Laboratory tests showed AFP at 9.15 ng/ml, CEA at 6.41 ng/ml, and CA 19-9 at 90.10 U/ml. The primary tumor and metastatic lesions had reduced by more than 30%, and the therapeutic effect was assessed as Partial Response (PR) according to the Response Evaluation Criteria in Solid Tumors (RECIST). Given the patient's young age and significant response to conversion therapy, he was readmitted for surgical treatment.

After multidisciplinary discussion and consultation, the patient underwent a pancreaticoduodenectomy and right hepatectomy under general anesthesia. Postoperative pathology indicated fibrous tissue proliferation, hyaline degeneration, and foam cell reaction in the "gray-white area" of the pancreaticoduodenectomy specimen, consistent with post-treatment appearance, with no definitive cancer residue, and surrounding tissue showing chronic inflammatory changes (Figures 2A, B). The liver specimen showed numerous mucinous lakes with no definitive cancer components, also consistent with post-treatment appearance (Figures 2C, D). Table 1 shows the lab results for pre and post treatment. The patient was followed up for two years, and no metastasis or recurrence was observed during the latest review (Supplementary Figure S1C). Supplementary Figure S2 shows the treatment timeline of this patient.

Case 2

A 48-year-old male was admitted to our department with abdominal distension, accompanied by skin and scleral icterus for over a month, and had undergone percutaneous transhepatic biliary drainage for two weeks. An enhanced abdominal CT scan revealed a slightly low-density mass in the pancreatic head, with blurred edges and a maximum cross-sectional area of approximately 4.2 x 3.4 cm. The enhancement was less than that of the pancreatic parenchyma, and the mass encircled the superior mesenteric vessels, causing significant stenosis of the superior mesenteric vein, slight thickening of the lower branches, and a rough edge of the superior mesenteric artery. The lesion was indistinct from the duodenum, with mild dilation of the main pancreatic duct, suggesting the possibility of pancreatic cancer with suspected invasion of the superior mesenteric vein (Supplementary Figure S3A). He had no significant past medical history and no relevant family history.

After multidisciplinary discussion, curative surgery was deemed unfeasible. A laparoscopic biopsy of the pancreatic mass was performed, and intraoperative findings included no nodular changes on the liver surface, a small amount of abdominal

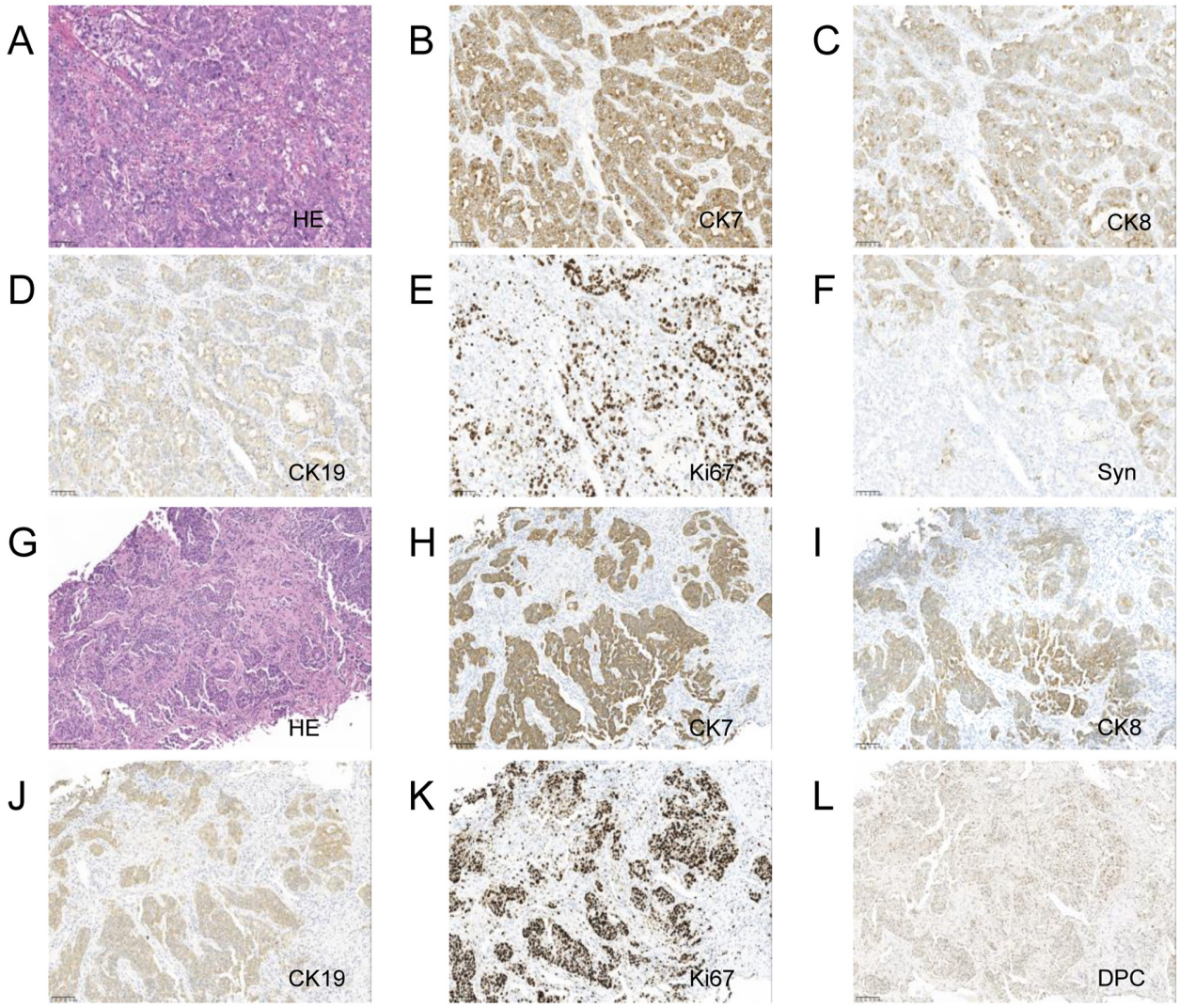

FIGURE 1
Pancreas and liver biopsy 20x light microscope image of case 1. **(A–F)** respectively shows 20x light microscope images of pancreas biopsy tissues HE, CK7, CK8, CK19, Ki67, Syn. **(G–L)** respectively shows 20x light microscope images of liver biopsy tissues HE, CK7, CK8, CK19, Ki67, DPC.

effusion, and a hard mass protruding from the head of the pancreas. The biopsy of the mass indicated atypical glands, suggesting adenocarcinoma (Figure 3). Genetic testing for immune-related markers showed MSI-H and a tumor mutational burden (TMB) of 3.84 (low). Somatic mutations were identified in genes including BRCA1, BRCA2, BRIP1, CHD1, EPHA7, ERCC3, ETV1, FANCD2, INPP48, LRP18, MSH3, PIK3C2G, PTEN, RASA1, SMAD2, STAT58, and TAP2.

The patient underwent his first FOLFIRINOX chemotherapy session on June 24, 2021, followed by a total of 10 sessions, but discontinued chemotherapy due to side effects, with the last session on October 22, 2022.The specific medication regimen (based on a body surface area of 1.90 m^2) was as follows: oxaliplatin 80 mg/m^2, irinotecan 170 mg/m^2, calcium folinate 315 mg/m^2, and fluorouracil 400 mg/m^2 administered on day 1 via intravenous infusion, followed by fluorouracil 4250 mg administered continuously by micro-pump over 46 hours. During the same period, the patient received immunotherapy with Camrelizumab 200 mg every three weeks (q3w). Due to the patient's inability to tolerate the side effects of chemotherapy and reluctance to continue, they received 14 cycles of immunotherapy alone from January 20, 2022, to October 22, 2022.

Enhanced CT scans were conducted every three months during the treatment period, showing continuous tumor regression. Initially, the tumor encircled the superior mesenteric artery (SMA) by more than 270 degrees (Supplementary Figure S3A). By June 2023, enhanced abdominal CT scans indicated that the tumor's contact with the SMA had reduced to less than 90 degrees (Supplementary Figures S3B-D). The patient's physical condition improved significantly, with complete resolution of jaundice, disappearance of abdominal pain, and weight gain. Surgical treatment was recommended, but the patient refused. The patient declined surgery, but the disease progressed, leading to four additional cycles of immunotherapy with Camrelizumab 200 mg every three weeks (Q3W) from October 13, 2023, to December 15, 2023. The patient underwent PTCD for jaundice on November 10,

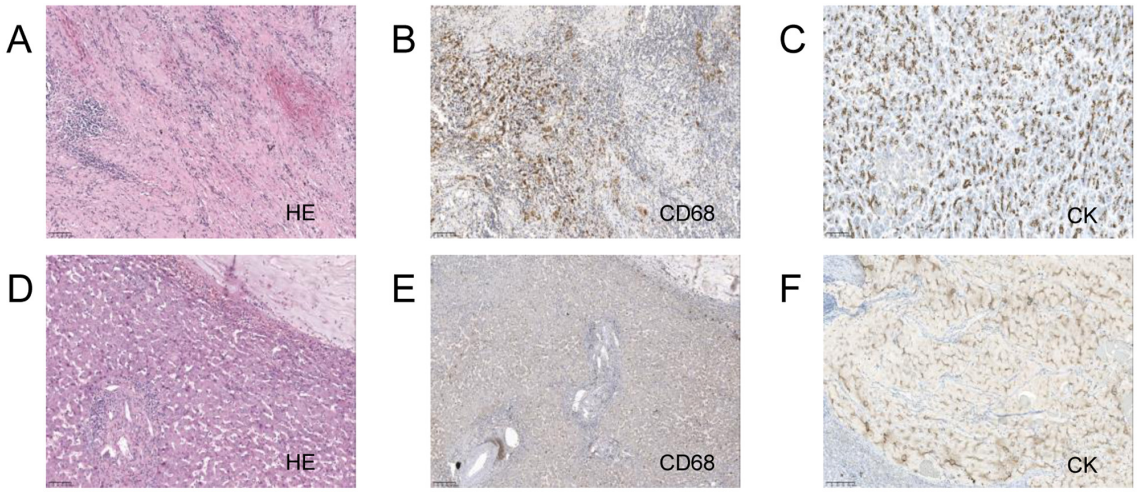

FIGURE 2
Pancreaticoduodenectomy and right hepatectomy tissue 20x light microscope image of case 1. **(A–C)** respectively shows 20x light microscope images of pancreas tissues HE, CD68, CK. **(D–F)** respectively shows 20x light microscope images of liver tissues HE, CD68, CK.

2023. On December 29, 2023, under general anesthesia, the patient underwent laparoscopic gastrojejunostomy, Roux-en-Y hepaticojejunostomy, cholecystectomy, and adhesiolysis. From April 25, 2024, to August 15, 2024, the patient received five cycles of Atezolizumab 1200 mg every three weeks (Q3W). Supplementary Figure S4 shows the treatment timeline of this patient.

Discussion

Pancreatic cancer is an aggressive and prevalent malignancy of the digestive tract, characterized by the highest mortality and lowest survival rates among all digestive tract cancers, with a 5-year survival rate of only 13% (1). Due to its deep anatomical location and the absence of early diagnostic methods, 70% to 80% of pancreatic cancer patients are diagnosed at a locally advanced or metastatic stage, with only 10% suitable for surgical resection (5, 6). Previous studies have shown that pancreatic cancer patients with liver metastasis generally have a total survival period not exceeding six months, even with active treatment (7).

Following treatment with chemotherapy and immunotherapy, the Case 1 patient have been proved complete response by postoperative pathology, and the patient has remained disease-free for over 24 months. We attribute the favorable treatment outcome in this case to the patient's genetic profile, which revealed multiple gene mutations, particularly BRCA mutations. Studies have demonstrated that patients with BRCA mutations are sensitive to platinum-based chemotherapy (8), leading us to employ a chemotherapy regimen of gemcitabine and cisplatin. Furthermore, the cut off value of high TMB are varies among studies. It is 10 mutations/Mb in Quintanilha's study (9). However, 5 mutations/Mb is the cut off value of high TMB in Hatakeyama (10) and Imamura studies (11). Therefore, we describe this case with a high TMB (9.84 mutations/Mb). Due to previous study confirmed that high TMB is associated with sensitivity to immunotherapy (9), we incorporated a PD-1 immune checkpoint inhibitor into the treatment.

In another advanced case with BRCA mutations, the patient exhibited low TMB and MSI-H, an indicator of sensitivity to immunotherapy (12). Consequently, we administered a treatment regimen of FOLFIRINOX plus PD-1, which resulted in significant tumor regression, resolution of jaundice, and substantial improvement in quality of life. Although the patient ultimately refused surgery and the disease progressed, a progression-free survival of 36 months was achieved. While MSI-H is often associated with a high TMB due to the accumulation of

TABLE 1 Lab results for pre and post treatment.

	Case 1		Case 2	
	Pre	Post	Pre	Post
ALT IU/L	23	74	123	64
AST IU/L	22	47	49	38
CA125 U/ml	1451	14.9	13.2	8.03
CA199 U/ml	>1000	53	19.2	10.5
CEA ng/ml	200	3.95	2.73	2.96
AFP ng/ml	7.46	9.19	6.12	6.36
TB umol/L	56.6	22.1	42.6	10.5
HB g/L	74	111	158	168
WBC 10^9/L	7.4	1.94	8.63	8.81
PLT 10^9/L	179	70	220	198

ALT, Alanine aminotransferase; AST, Aspartate transaminase; CA125, Carbohydrate antigen 125; CA199, Carbohydrate antigen 199; CEA, Carcinoembryonic antigen; AFP, Alpha-fetoprotein; TB, Total bilirubin; HB, Hemoglobin; WBC, White blood cell; PLT, Blood platelet.

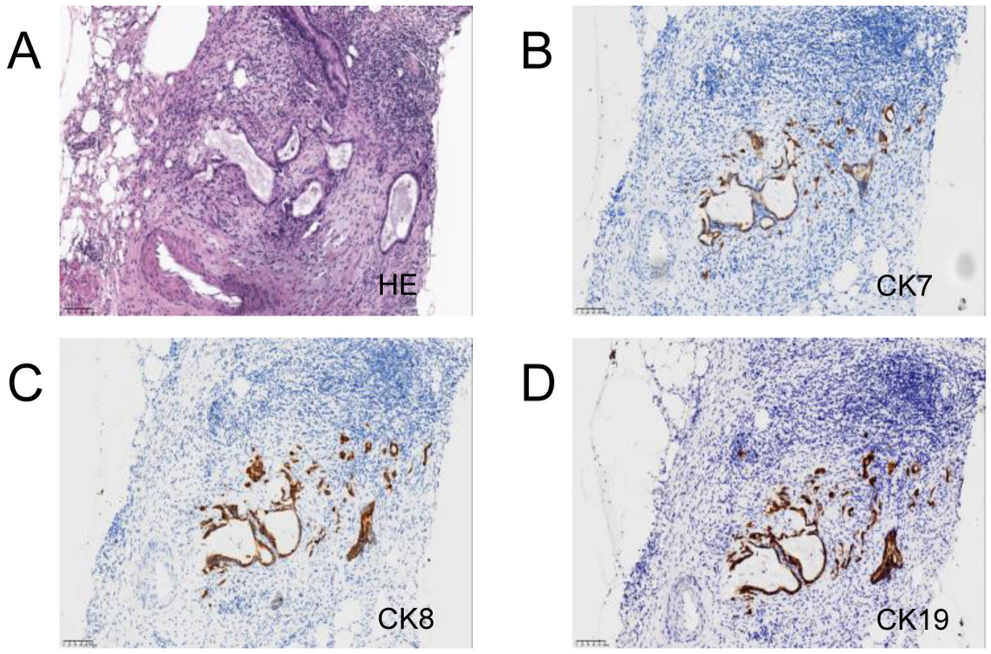

FIGURE 3
Pancreas and liver biopsy 20x light microscope image of case 2. **(A–C)** respectively shows 20x light images of pancreas tissues HE, CD68, CK. **(D–F)** respectively shows 20x light images of liver tissues HE, CD68, CK.

mutations from mismatch repair deficiencies. Some MSI-H tumors may exhibit a low TMB, potentially due to specific genetic mutations or unique tumor biology. MSH3, a component of the mismatch repair (MMR) system, a major source for the inactivation in MSI frameshift events (13). Mutations in MSH3 can lead to MSI-H. However, the impact on TMB might be more nuanced. It is possible that MSH3 mutations result in selective repair deficiencies, affecting only certain types of DNA errors while not significantly increasing the overall mutation burden. Additionally, other compensatory repair mechanisms may mitigate the expected increase in TMB despite the presence of MSI-H.

Previous clinical studies have shown that the overall efficacy of immunotherapy in treating pancreatic cancer is limited. For example, the overall response rate of PD-1 antibody monotherapy or combined CTLA-4 antibody therapy is only 0.10%-12% and 3%, respectively (3, 4, 11–15). Despite numerous clinical trials on immune checkpoint inhibitors, monoclonal antibodies against key antigens, and immune cell therapies, satisfactory clinical benefits have not been realized. The immune microenvironment of pancreatic cancer is highly heterogeneous, posing significant challenges to immunotherapy (2). However, some patients can still benefit from immunotherapy, particularly those with MSI-H or high TMB. A study by the University and Hospital Trust of Verona analyzed data from 8,323 patients with pancreatic adenocarcinoma, finding that only 1% and 2% of patients had MSI-H and high TMB, respectively (16).

BRCA mutations are associated with defects in the DNA repair pathway, potentially increasing the mutation burden in tumor cells. Therefore, BRCA mutations may have potentially pathogenic in both cases. Some studies indicate that the combination of high TMB with BRCA mutations may lead to immune activation within the tumor microenvironment, characterized by higher levels of T lymphocyte infiltration and increased PD-L1 expression (17). These factors may enhance the efficacy of immunotherapy. Both patients significant responded to the combination of chemotherapy and immunotherapy in present study may as evidence for that.

A recent report from Memorial Sloan Kettering Cancer Center indicates that patients with BRCA2 mutations are more responsive to immune checkpoint blockade (ICB). Interestingly, this benefit is observed primarily in patients with tumors not typically rich in homologous recombination deficiency (HRD), such as melanoma and small cell lung cancer, whereas patients with HRD-related tumors (breast, prostate, pancreatic, or ovarian cancers) did not benefit as much from ICB (18).

Additionally, we know that it is difficult to determine the role of immunotherapy in the clinical outcome of two cases with different MSI and TMB statuses. However, both of them were responded well to chemotherapy combined with immunotherapy. One patient had MSI-H with low TMB, while the other had high TMB with stable microsatellites. This phenomenon aligns with findings that BRCA mutations rarely occur in the background of MSI-H but are more common with high TMB (17). This observation is also related to WRN gene mutations, which are closely associated with high TMB (19). In our reported cases, one patient had a WRN mutation, while the other did not. Despite the different MSI and TMB statuses, both patients achieved favorable outcomes with chemotherapy and immunotherapy. Therefore, we suggest that screening pancreatic cancer patients for MSI-H or high TMB may be an effective strategy for identifying candidates for immunotherapy.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by Ethics committee of West China Hospital. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study. The manuscript presents research on animals that do not require ethical approval for their study. Written informed consent was obtained from the individual (s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

HL: Formal analysis, Investigation, Software, Validation, Writing – original draft. YW: Formal analysis, Investigation, Methodology, Writing – original draft. QZ: Data curation, Investigation, Methodology, Writing – review & editing. NK: Data curation, Formal analysis, Investigation, Project administration, Supervision, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2024.1458517/full#supplementary-material

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Case report: PD-L1-targeted high-affinity natural killer cells and IL-15 superagonist N-803-based therapy extend overall survival of advanced metastatic pancreatic cancer patients

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Background: Metastatic pancreatic cancer (mPC) is an aggressive form of cancer with a poor prognosis and few therapeutic options after failure of the second-line treatment. Induction of immunogenic cell death (ICD) by use of relatively low-dose chemo- or radiation therapy, enhancement of immune responses by the IL-15 superagonist N-803 (Anktiva®), and targeting of programmed death receptor ligand 1 (PD-L1)-expressing cells may offer a therapeutic approach to refractory mPC with the potential to increase overall survival (OS).

Methods: From late 2019 to 2021, single-patient Investigational New Drug (spIND) protocols for five mPC patients were designed and approved that generally comprised combined Abraxane (nab-paclitaxel) and gemcitabine therapy with experimental therapeutics N-803, PD-L1-targeted high-affinity natural killer (PD-L1 t-haNK) cells, and aldoxorubicin, a serum albumin-binding doxorubicin prodrug. Some patients also received stereotactic body radiation therapy (SBRT), cyclophosphamide, pembrolizumab, nivolumab, and/or experimental ETBX-051 (brachyury) and/or ETBX-061 (MUC1) vaccines. Duration of spIND treatment and responses, for some patients including imaging and carbohydrate antigen 19-9 (CA19-9) levels, and OS from initial diagnosis and the start of spIND therapy were assessed.

Findings: The line/duration of spIND therapy was, for patients 1 through 5, respectively, second line/6.4 months, sixth line/3.5 months, third line/25.4 months, third line/7.4 months, and fourth line/23.2 months. OS from the commencement of spIND therapy was 13, 4.8, 26.9, 9, and 23.2 months, and OS from diagnosis was 22, 21, 42, 13, and 33 months for patients 1 through 5, respectively.

Conclusions: The OS from the initiation of spIND for all patients exceeded the reported OS for the greater-than-second-line mPC patients and, for four of five patients, second-line therapy. The OS of 13, 26.9, and 23.2 months for three patients is particularly notable. The findings here support the ongoing clinical investigations of N-803 and PD-L1 t-haNK cells in combination therapy.

KEYWORDS

advanced metastatic pancreatic cancer, 3rd line therapy, orchestrated, multi-modal, N-803, PD-L1 t-haNK cells, low dose chemotherapy

Introduction

Pancreatic cancer has one of the highest rates of mortality worldwide (1) and is anticipated to become the second most common cause of death related to cancer by 2030 (2). At diagnosis, the 5-year survival rate of patients with metastatic pancreatic cancer (mPC) is only 14.4% for locally advanced disease and only 3% for those with distant metastases (3, 4); this rate decreases further for patients for whom first- and second-line therapies fail. Little survival data are available for third-line therapy, but as reported by Nagrial et al. (5) in their systematic review of locally advanced or mPC patient response to second-line treatment, the median overall survival (OS) was 4.0–5.4 months.

The current standard of care (SoC) for mPC includes FOLFIRINOX or Nab-paclitaxel plus gemcitabine as first-line therapy and crossover to Nab-paclitaxel/gemcitabine or FOLFIRINOX as second-line therapy (6–8). Nanoliposomal irinotecan with 5-fluorouracil (5-FU) and leucovorin is also approved as second-line therapy after gemcitabine therapy, based on the findings from the phase 3 NAPOLI 1 trial wherein the median survival was 6.1 months (9, 10). These treatment regimens are typically alternated for patients whose status allows for continued therapy and who do not opt for palliative care only.

There are little published data on OS after third-line therapy. In a study performed in Japan, an OS of 4.6 months for erlotinib plus gemcitabine as the third-line or greater therapy for mPC was reported (11). In a retrospective analysis of the efficacy of second-line or greater therapy for mPC patients, Bachet et al. reported that median OS was 4.6 months after first-line therapy only and 11.5 months from diagnosis and 4.7 months from initiation of second-line therapy for patients receiving two or three lines of therapy (12). These data highlight the unmet need for new therapeutic options for mPC patients beyond second-line therapy.

Here, as an alternative therapeutic approach targeted to induce the death of cancer cells, we sought to enhance immunogenic cell death (ICD). In the tumor microenvironment (TME), ongoing expression of damage-associated molecular patterns (DAMPs) should, in the presence of an effective immune response, elicit the release of ligands and expression of dendritic cell (DC) receptors that facilitate antigen processing and presentation, ultimately stimulating the anti-tumor cell cytotoxicity of T cells (13) and natural killer (NK) cells (14), with the goal of establishing immune memory (Figure 1).

Frequently, in the case of an ineffective immune response to neoplastic growth, expression of receptors such as the programmed death receptor 1 (PD-1) or its ligand (PD-L1) by immune cells in the TME circumvent an effective immune response and allow tumor cells to proliferate unabated. To re-initiate immune activity, inhibitors of PD-1 or PD-L1 (checkpoint inhibitors) are used.

In the therapeutic protocols described here, we used ImmunityBio's investigational PD-L1-targeted high-affinity NK cells (PD-L1 t-haNK), a novel NK cell line that are NK-92 cells engineered to express high-affinity CD16, endoplasmic reticulum-retained interleukin (IL)-2, and a PD-L1-specific chimeric antigen receptor (CAR) (15, 16).

To further enhance the immune response, ImmunityBio's interleukin-15 (IL-15) superagonist N-803 (nogapendekin alfa inbakicept, Anktiva®; previously known as ALT-803) (17), a human IL-15 variant bound to a dimeric human IL-15 receptor α (IL-15Rα) sushi domain/human IgG1 Fc fusion protein, was added. It targets both the innate and adaptive immune system, acting as a growth and activation factor for NK cells as well as effector and memory T cells (18, 19). N-803 alone or in combination with an anti-PD-L1 antibody has been shown to elicit robust anti-tumor immune responses and prolonged survival in tumor-bearing mice (20, 21). N-803 has also been shown to increase PD-L1 expression both *in vitro* (22) and in breast tumor-bearing mice (23); it has been suggested that this may allow lymphocytes to become targets of anti-PD-L1 therapy.

To induce the release of DAMPs, low/moderate dose nab-paclitaxel (Abraxane), paclitaxel bound to albumin that has a better safety profile than paclitaxel and an improved response rate, was used typically several days before the introduction of the PD-L1 t-haNK cells and N-803. Abraxane is used to treat non-small cell lung cancer (NSCLC), breast cancer, and pancreatic cancer and is being studied in other cancers (24–26). Abraxane given together with anti-PD-L1 therapies such as atezolizumab has been reported to prolong

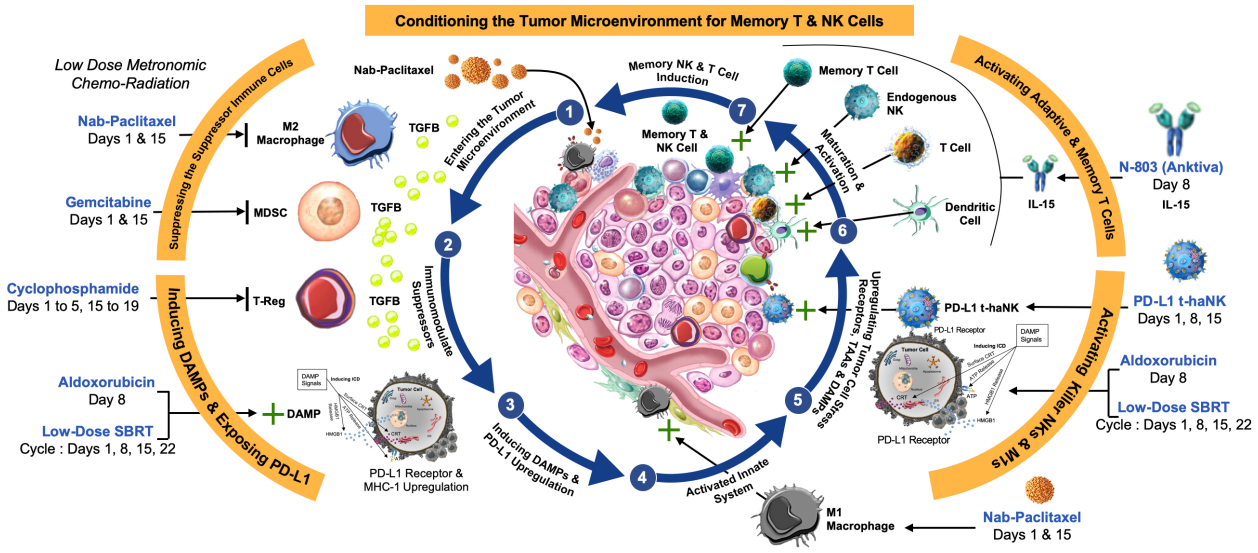

FIGURE 1
Rationale for multimodal therapy to establish immune memory. (1) Low-dose chemotherapy such as Nab-paclitaxel (Abraxane) and stereotactic body radiation therapy (SBRT) is anticipated to (2) reverse immunosuppression by M2 macrophages, myeloid-derived suppressor cells (MDSCs), and regulatory T cells (Tregs) in the tumor microenvironment (TME). (3) The release of damage-associated molecular patterns (DAMPs) from dying tumor cells leads to upregulation of MHC-1, and often PD-1/PD-L1 expression, (4) activating the innate immune system. (5) Further upregulation of cell stress receptors and tumor antigens by aldoxorubicin and SBRT also contributes to activation of M1 macrophages, cytotoxic T, and natural killer (NK) cells, an effect that is enhanced by (6) the IL-15 superagonist N-803. PD-L1-targeted high-affinity NK (PD-L1 t-haNK) cells target and reverse immunosuppression. The goal is (7) dendritic cell (DC) maturation and activation, leading to establishment of memory T cells.

progression-free survival among patients with metastatic triple-negative breast cancer (mTNBC) (27) and NSCLC (28). When used at moderate or relatively low doses, Abraxane treatment has the potential to result in the release of tumor cell-associated antigens (TAAs) and DAMPS that may enhance the effects of immunotherapy to elicit a vaccine-like anti-tumor response (29).

Additional experimental chemotherapy was also utilized for the patients described here—aldoxorubicin (ImmunityBio), a doxorubicin prodrug that binds serum albumin post-administration through an acid-sensitive hydrazone linker (30). It leverages the relatively acidic environment of solid tumors that facilitates cleavage of the linker and tumor-targeted delivery of doxorubicin. Aldoxorubicin has shown clinical efficacy and mitigated cardiac toxicity (31–33). In general, for the single-patient Investigational New Drug (spIND) patients here, it was given on the day of PD-L1 t-haNK/N-803 administration.

Some of the patients also received adenovirus vector-based vaccines, including ETBX-051 (Etubics/ImmunityBio), a brachyury vaccine that after subcutaneous administration expresses brachyury that is predicted to elicit a cytotoxic T lymphocyte (CTL)-mediated immune response against tumor cells expressing brachyury and ETBX-061, which, like ETBX-051, is an adenovirus vector-based vaccine that expresses the transmembrane mucin MUC1 (34) after subcutaneous delivery.

Other therapeutics, including cyclophosphamide (an alkylating agent), capecitabine (Xeloda®; an anti-metabolite), nivolumab (anti-PD-1), pembrolizumab (anti-PD-1), cisplatin (DNA crosslinker), denosumab (anti-RANKL (35)), and stereotactic body radiation therapy (SBRT; an inducer of DAMP release), were given to individual patients in some instances.

A description of the putative role of each agent in therapy and the manufacturer of each agent are shown in Supplementary Table S1.

Case report 1

Patient 1 (Pt 1), a 62-year-old man, was diagnosed with mPC in May 2019 (Supplementary Table S2) and underwent 11 cycles of first-line FOLFIRINOX therapy (Figure 2), a four-drug combination of leucovorin (folinic acid), fluorouracil, irinotecan HCl, and oxaliplatin (36, 37). Initial treatment of this patient was complicated by diarrhea and grade 2 neuropathy. Several months later, the patient received three cycles of FOLFIRINOX as therapy, after which disease progression was observed by PET/CT in February 2020 (Supplementary Table S2).

As second-line therapy, a spIND protocol was designed, approved, and consented to by the patient, which commenced in March 2020 and comprised alternating IV gemcitabine (600 mg/m^2) plus Abraxane (75 mg/m^2) with IV infusion of 2×10^9 PD-L1 t-haNK cells and 15 µg/kg N-803 by subcutaneous (sc) injection (Figure 2). The doses of gemcitabine and Abraxane, respectively, were increased to 750 mg/m^2 and 100 mg/m^2 from April 14 to June 22, 2020, and to 1,000 mg/m^2 and 125 mg/m^2 on July 14 and 21, 2020. Neutrophil-to-lymphocyte (N/L) ratios varied throughout treatment with a high value recorded on March 13, 2020; the

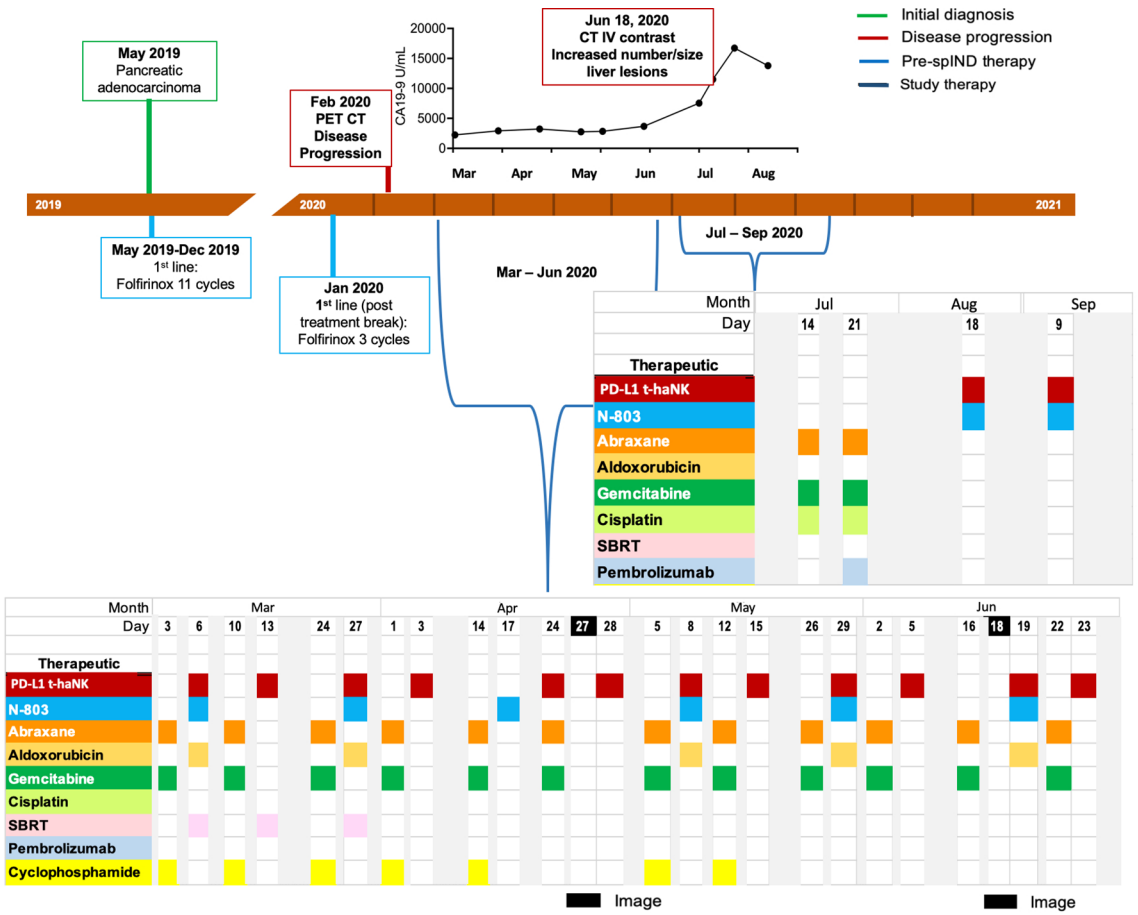

FIGURE 2
Metastatic pancreatic cancer (mPC) single-patient Investigational New Drug (spIND) patient 1 diagnosis and treatment history. mPC diagnosis (green box) in May 2019 was followed by first-line FOLFIRINOX treatment. Disease progression (red box) detected in February 2020 prompted initiation of a spIND protocol comprising Abraxane (orange), gemcitabine (dark green), cyclophosphamide (light yellow), PD-L1 t-haNK (red), N-803 (aqua), stereotactic body radiation therapy (SBRT; pink), aldoxorubicin (dark yellow), cisplatin (light green), and pembrolizumab (light blue) given on the days indicated. Dates of imaging are shown in black. CA19-9 levels (U/mL) from March to August 2020 are shown above the timeline.

highest absolute neutrophil count (ANC) was observed at baseline on March 3, 2020, and at the last assessment in August 2020 (Supplementary Table S3). PET/CT indicated stable disease (SD) in April 2020 (Table 1, Supplementary Table S2). Along with the first four infusions, the patient received stereotactic body radiation (X-ray) therapy per the schedule shown in Figure 2. Aldoxorubicin (80 mg/m^2) was also given on some days of PD-L1 t-haNK infusion/N-803 delivery and cyclophosphamide (25–50 mg, PO) on some days of Abraxane/gemcitabine delivery. In June, CT indicated progressive disease (PD) (Supplementary Table S2). In July 2020, the patient received Abraxane, gemcitabine, and cisplatin (25 mg/m^2); he reported fevers, rigors, and nausea post-therapy. A single dose of pembrolizumab (200 mg IV) was administered on July 21. In August and September 2020, the patient received PD-L1 t-haNK and N-803 only. CA19-9 levels from March 2020 to August 2020 are shown in Figure 2 and increased after June 2020, peaking in July 2020. Abraxane and gemcitabine doses were reduced in August due to neutropenia. The patient received spIND treatment for ~6.4 months. The patient's date of death was March 31, 2021; OS from diagnosis was 22 months, and OS from initiation of spIND therapy was 13 months (Table 1).

Case report 2

Patient 2 (Pt 2), a 78-year-old man, was diagnosed with stage IV pancreatic adenocarcinoma in February 2019 (Supplementary Table S2) and received gemcitabine and Abraxane as first-line treatment (Figure 3). Due to the toxicity of first-line therapy, the patient was switched to pembrolizumab as second-line therapy in July 2019. In response to increasing CA19-9 levels in September 2019, gemcitabine and Abraxane therapy was reinstated. In December 2019, the patient was given an experimental regimen of SM-88 with methoxsalen, Dilantin, Rapamune, and sirolimus as fourth-line therapy. CA19-9 levels were relatively elevated in January 2020, and a CT scan revealed progressive disease (Supplementary Table S2); the patient began treatment with a combination of gemcitabine and cisplatin.

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TABLE 1 Data overview for all patients.

Case study patient #	Line of therapy	Duration of spIND therapy (mo)	Survival from diagnosis	Survival from start of spIND therapy (mo)	Best response	Adverse events during spIND therapy
1	2	6.4	22	13	Stable disease	Post-chemotherapy fever, rigor, nausea, and neutropenia
2	6	3.5	21	4.8	Disease response*	Abdominal distension
3	3	25.4	42	26.9	Biochemical disease response**	Neutropenia, post-chemotherapy fever
4	3	7.4	13	9	Disease response*	Neutropenia, anemia
5	4	23.2	33	23.2	Complete response	Fatigue, bilateral leg edema, neutropenia, anemia

spIND, single-patient Investigational New Drug (regimen); mo, months.
*Disease response, reduction in target lesion size, not necessarily meeting Response Evaluation Criteria in Solid Tumors (RECIST) criteria for a defined partial response.
**Disease response suggested by decreased CA19-9 levels.

In June 2020 as sixth-line therapy, a spIND protocol comprising PD-L1 t-haNK (IV infusion 2×10^9 cells/dose), N-803 (15 µg/kg sc), and aldoxorubicin (150 mg/m^2 IV) alternating with IV gemcitabine (300 mg/m^2) and Abraxane (50 mg/m^2) was initiated with the patient's consent, with therapeutics delivered as shown in Figure 3. The lowest ANC was observed at baseline in June 2020 and the highest at the last assessment in September 2020; N/L values were lower throughout treatment relative to baseline (Supplementary Table S4). Nivolumab (480 mg) was added and gemcitabine/Abraxane was withheld starting in September 2020. During the course of spIND therapy, the patient continued to report abdominal distension. The patient received spIND treatment for 3.5 months. In August 2020, a CT scan showed some disease response (Table 1, Supplementary Table S2) at the same time CA19-9 levels were low; disease progression and CA19-9 level increases were seen in September 2020 (Figure 3, Supplementary Table S2). The

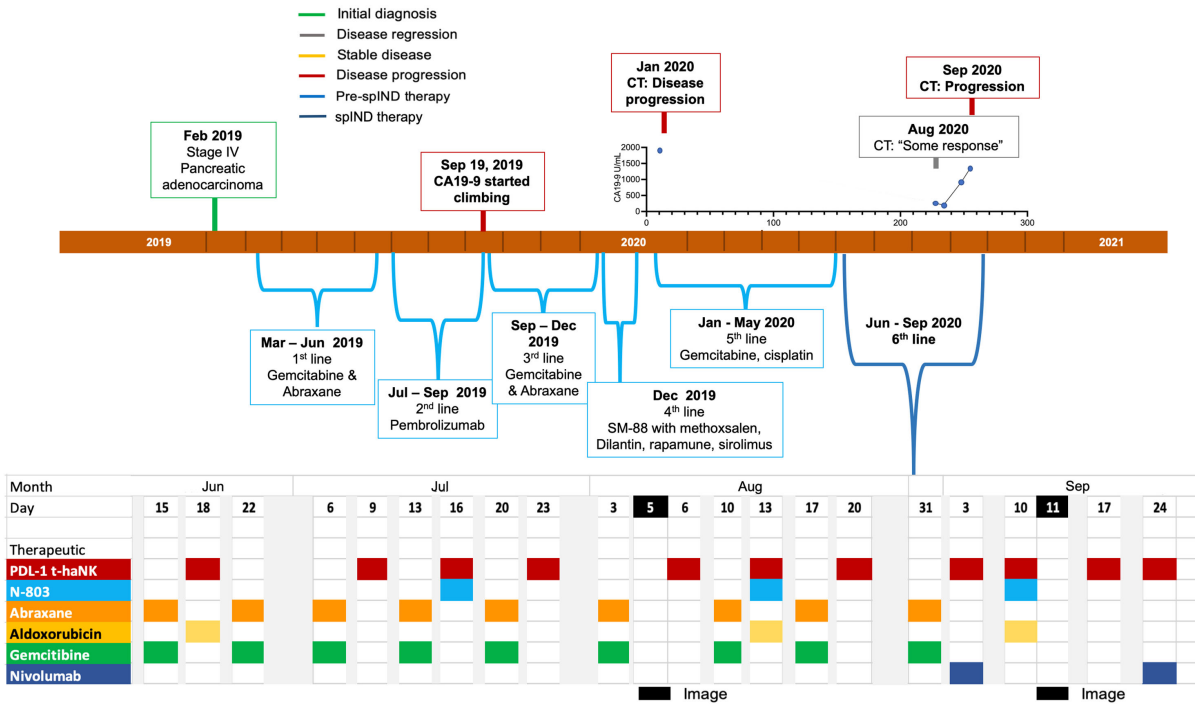

FIGURE 3
Metastatic pancreatic cancer (mPC) single-patient Investigational New Drug (spIND) patient 2 diagnosis and treatment history. mPC diagnosis (green box) in February 2019 was followed by first-, second-, third-, fourth- (experimental SM-88 therapy), and fifth-line therapies up to June 2020, at which time the spIND protocol commenced comprising Abraxane (orange), gemcitabine (green), PD-L1 t-haNK (red), N-803 (blue), aldoxorubicin (yellow), and nivolumab (dark blue) given on the days indicated. There was evidence of disease progression (red boxes) and some response (gray box) on the dates shown. Dates of imaging are shown in black. CA19-9 levels (U/mL) in January 2020 and from August to September 2020 are shown above the timeline.

patient's date of death was November 8, 2020. The patient received spIND therapy for 3.5 months, survival from diagnosis was 21 months, and survival from initiation of spIND therapy was 4.8 months (Table 1).

Case report 3

Patient 3 (Pt 3), a 78-year-old man, was diagnosed with grade III pancreatic adenocarcinoma in May 2018 (Supplementary Table S2) at which time he underwent a distal pancreatectomy and splenectomy. First-line therapy from July to October 2018 was FOLFIRINOX with Neulasta (Supplementary Figure S1). A PET/CT in May 2019 revealed metastases (Supplementary Table S2); CA19-9 levels also rose from ~40 U/mL in February 2019 to 4,484 U/mL in August 2019. As second-line therapy, Abraxane and gemcitabine were given between July and September 2019. spIND therapy was initiated in November 2019 with the patient's consent and comprised PD-L1 t-haNK (2×10^9 cells/infusion IV), aldoxorubicin (80 mg/m^2), cyclophosphamide (50–75 mg PO), N-803 (15 µg/kg sc), Abraxane (75 mg/m^2), and gemcitabine (600 mg/m^2) with a single instance of denosumab (60 mg) administration up to the end of 2019 (Supplementary Figure S1).

In December 2019, the chemotherapeutic agents were held due to neutropenia.

The patient's therapy continued from January to June 2020, including the therapeutics described above, as well as a single dose of capecitabine (500 mg PO) (Supplementary Figure S2). In February 2020, immunotherapy was held due to fever post-chemotherapy, and in April 2020, treatment was delayed for ANC. The dose of aldoxorubicin was decreased to 60 mg/m^2 in March. Abraxane and gemcitabine were held for several months starting in May. CA19-9 levels over this treatment period remained under 1,000 U/mL (Supplementary Figure S2). N/L and ANC values varied throughout therapy, as shown in Supplementary Table S5.

Beginning in late August 2020, nivolumab (480 mg IV) was added to the therapeutic arsenal for patient 3, and Abraxane/gemcitabine was re-introduced in October 2020 (Figure 4). Also in October 2020, the patient received vaccination with ETBX-051/ETBX-61 [both 1×10^{11} viral particles (VP) sc], and dosing with capecitabine (500 mg PO) became more frequent. This regimen continued until October 2021 and included a single administration of cisplatin (25 mg/m^2) in May 2021 and a single instance of SBRT in July 2021. As shown in Figure 4, CA19-9 levels surpassed the 1,000 U/mL level in September and remained steady until May/June, at which time they began to rise. This increase was associated with evidence of progressive disease (Supplementary Table S2). The patient received 25.4 months of spIND treatment (up to October 2021), after which he received palliative care and died in January 2022. He survived 42 months from the time of diagnosis and ~27 months from initiation of spIND therapy (Table 1).

Case report 4

Patient 4 (Pt 4), a 59-year-old woman, was diagnosed with pancreatic adenocarcinoma in November 2019 (Supplementary Table S2) and received first-line FOLFIRINOX plus CPI from November 2019 to February 2020 treatment, during which time (January 2020) pancreatic disease appeared stable (Supplementary Table S2) but suspicious liver lesions were detected. In March 2020, second-line treatment with 5-FU, gemcitabine, and Abraxane was given (Supplementary Figure S3). In April 2020, with the patient's consent, third-line spIND treatment was initiated with PD-L1 t-haNK (4×10^9 cells IV), N-803 (15 µg/kg sc), gemcitabine (600 mg/m^2), and Abraxane (100 mg/m^2 starting; then decreased to 75 mg/m^2) with two rounds of SBRT, intermittent cyclophosphamide (50 mg PO), and aldoxorubicin administration (150 mg/m^2); and addition of nivolumab (480 mg) in September 2020 (Figure 4). The highest N/L ratio and ANC were recorded near the end of spIND therapy (Supplementary Table S6). Some response was observed in June 2020, but new liver metastases were observed in October 2020 (Supplementary Table S2). The patient received single doses of ETBX-05 and ETBX-061 vaccines (1×10^{11} VP sc) in November. Therapy lasted for 7.4 months and continued until December 2020. Over the last 6 months of therapy, the patient showed weight loss. The patient's date of death was January 21, 2021; OS from diagnosis was 13 months, and OS from initiation of spIND therapy was 9 months (Table 1).

Case report 5

Patient 5 (Pt 5), a 61-year-old woman, was diagnosed with pancreatic cancer in March 2019 (Supplementary Table S2), and from April to December 2019, she underwent first-line therapy with FOLFIRINOX, followed by second-line radiation therapy with Xeloda and third-line gemcitabine and Abraxane therapy, as shown in Supplementary Figure S4. Disease progression was detected by a laparoscopic look in December 2019 and a slight decrease in lesion size by PET/CT in January 2020 (Supplementary Table S2), at which time the highest ANC value was observed (Supplementary Table S7). As fourth-line therapy, spIND therapy was initiated in January 2020 with the patient's consent and comprised Day 1 gemcitabine (750 mg/m^2 starting, decreased to 600 or 300 mg/m^2 on some occasions, IV) and Abraxane (100 mg/m^2 starting and then decreased to 75 mg/m^2, IV) followed by Day 3 PD-L1 t-haNK cells (2×10^9 cells IV), N-803 (15 µg/kg sc), and aldoxorubicin (80 mg/m^2 starting and then decreased to 40–60 mg/m^2) on a 1-week cycle until September 2020. CA19-9 levels in July and August 2020 were 47 and 117 U/mL, respectively; PET/CT and CT with IV contrast suggested that the disease was stable during this period (Supplementary Table S2). Neutropenia was noted early in the therapeutic regimen (February 2020) and anemia in April 2020.

From October 2020 to December 2021 (Supplementary Figure S5), gemcitabine and Abraxane were not given, and dosing with other therapeutics was less frequent. ETBX-051/ETBX-061 vaccines (1×10^{11} VP sc) were added during this time period. In March 2021, a PET/CT revealed no evidence of active malignancy (Supplementary Table S2). In May 2021, regular ~2-week cycles of PD-L1 t-haNK with either the vaccines or aldoxorubicin were established, with the addition of N-803 in August through November. spIND therapy lasted 23.2 months. CA19-9 levels did not vary greatly from January

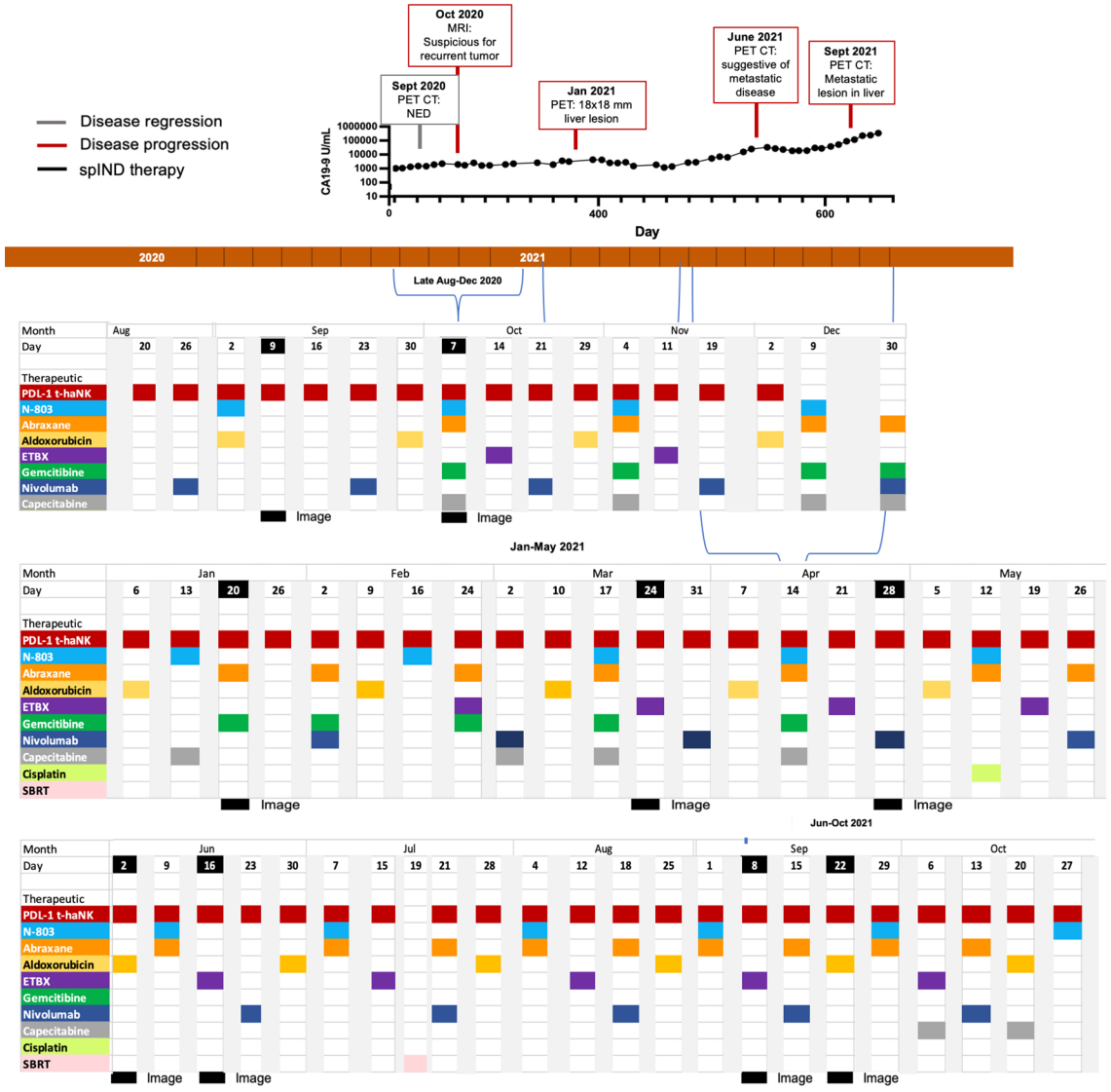

FIGURE 4
Metastatic pancreatic cancer (mPC) single-patient Investigational New Drug (spIND) patient 3 treatment history from late August 2020 until October 2021. The spIND protocol comprised PD-L1 t-haNK (red), N-803 (blue), Abraxane (orange), aldoxorubicin (yellow), ETBX vaccines 051 and 061 (purple), gemcitabine (green), nivolumab (dark blue), and capecitabine (gray), given on the dates shown; the patient also received a single dose of cisplatin (light green) and a single instance of stereotactic body radiation therapy (SBRT; pink). Disease regression is shown in gray boxes, disease progression in red boxes, and dates of imaging in black. CA19-9 levels (U/mL) from September 2020 to October 2021 are shown above the timeline.

to October 2021 and were low, ranging from 36 to 117 U/mL. Some fatigue was reported by the patient in October 2021 and bilateral leg edema. Disease progression—a liver lesion—was assessed by PET/CT in August 2021 (Supplementary Table S2). The patient was still alive as of the writing of this manuscript, with an OS from diagnosis of 33 months and OS from initiation of spIND therapy of 23.2 months (Table 1).

Discussion

We chose the unusual approach of presenting several case studies in a single report to enable better representation and understanding of the orchestrated, multi-modal therapy strategy employed for these advanced PC patients. Considered together, these case studies reveal the potential for this strategy to extend the survival of this patient group.

The OS of the five patients treated by orchestrated multi-modal nab-paclitaxel/gemcitabine, N-803, PD-L1 t-haNK cell, and aldoxorubicin-based therapy, whether from the commencement of spIND therapy (range 4.8 to 26.9 months) or diagnosis (range 13–42 months), is notable and highly clinically relevant (Table 1). Perhaps more remarkable is that therapy was the second line for only one of these patients, the third line for two, and the fourth or sixth line for one each.

Because the line of therapy was greater than second in four of five of the case studies here, it is difficult to compare OS to published reports, but if one considered studies of second-line therapy such as that of Tsang et al., a median OS of ~8 months was observed with

gemcitabine/nab-paclitaxel (38) in patients who were, as compared to those who did not receive second-line therapy, were younger, with lower Eastern Cooperative Oncology Group (ECOG), and with higher CA19-9 (at presentation). In another study (39), the median OS for FOLFOX as second-line therapy was 2.6–6.7 months, and that for 5-FU/leucovorin plus nanoliposomal irinotecan was ~6 months. In the sole second-line patient presented here, OS was 13 months from the start of spIND therapy and 22 months from diagnosis. The shortest OS here of 4.8 months from the start of spIND therapy was for the sixth-line patient.

The compassionate use of spIND therapy was well-tolerated, with patients reporting few adverse events. Those that were noted—fatigue, fevers, rigors, nausea, and abdominal distension—or based on laboratory assessments (neutropenia and anemia) were not unexpected and likely associated with chemotherapy.

While the individual spIND protocols were not designed so that regular assessments of tumor size or metastases would be evaluated by, for example, Response Evaluation Criteria in Solid Tumors (RECIST) criteria, imaging scans suggested that at some point during spIND therapy, stable disease was achieved in patient 1; disease response in patients 2, 3, and 4; and a complete response in patient 5 (Table 1). ANC levels and N/L ratios did not show a notable pattern among patients; although higher values are associated with decreased overall survival in cancer (40) and with the presence of metastases in pancreatic cancer (41), the relationship between these values and response to therapy is not fully understood.

The findings presented here provide support for larger trials of multi-modal therapy that includes PD-L1 t-haNK cells, N-803, and other therapeutics that play a role in inducing immunogenic cell death. Such clinical trials may be limited to patients who have failed first-line or greater therapy, but there may also be merit in determining the benefit in patients as first-line therapy. In such future studies, it would be of interest to collect pre-study tumor tissue biopsies for gene expression and other analyses to allow identification of the TME as well as tumor cell molecular and genetic factors that are associated with response, duration, or failure of this treatment regimen.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving humans were approved by Chan Soon-Shiong Committee; Western IRB. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

TS: Investigation, Methodology, Supervision, Writing – review & editing. LS: Conceptualization, Investigation, Methodology, Project administration, Resources, Supervision, Writing – review & editing. OJ: Data curation, Formal Analysis, Investigation, Visualization, Writing – review & editing. FJ: Data curation, Formal Analysis, Investigation, Writing – review & editing. PS: Data curation, Visualization, Writing – original draft, Writing – review & editing. SR: Conceptualization, Investigation, Methodology, Project administration, Resources, Supervision, Writing – review & editing. PS: Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Writing – review & editing.

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Conflict of interest

TS, LS, FJ, PS, SR, and PS-S are employees or shareholders of ImmunityBio, Inc., which is developing N-803, PD-L1 t-haNK cells, and the described vaccines. PS-S is a shareholder in Celgene, acquired by the manufacturer of Abraxane, Bristol Myers Squibb. FJ was an original developer of ETBX vaccines and was employed by NantCell.

The remaining author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2025.1472714/full#supplementary-material

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Efficacy and safety of immune checkpoint inhibitors in patients with advanced intrahepatic cholangiocarcinoma

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Background: To assess the efficacy and safety of PD-1 and PD-L1 immune checkpoint inhibitors (ICIs) in managing advanced intrahepatic cholangiocarcinoma (ICC).

Methods: A retrospective analysis of treatment data for patients with advanced ICC who received ICIs at the Second Affiliated Hospital of Chongqing Medical University from the inception of the inpatient medical record database until 30 April 2024. The analysis concentrated on the safety and efficacy of the treatment. The primary endpoint was progression-free survival (PFS), while the secondary endpoints included overall survival (OS) and safety. The Kaplan-Meier method was employed to plot survival curves, and differences between groups were assessed using log-rank tests.

Results: 96 patients diagnosed with ICC were included, comprising 60 males (62.50%) and 36 females (37.50%). 85 patients exhibited disease progression, 22 patients succumbed, and 38 patients were lost to follow-up finally. Those who initiated immunotherapy promptly following first-line antitumor treatment exhibited a notably prolonged PFS compared to those experiencing tumor progression (5.63 months (*95%CI:* 3.12~8.14) vs 2.50 months (*95%CI:* 1.83~3.17), P=0.002). However, no significant disparity in the PFS with immunotherapy in different lines therapy(P=0.406) and the OS was observed between the two groups(P=0.360). 18 patients (18.75%) experienced treatment-emergent adverse events (AEs), with 3 patients encountering AEs of grade ≥3. All patients returned to normal after symptomatic treatment.

Conclusions: In patients with advanced ICC, the timely initiation of ICIs as adjuvant therapy following first-line antitumor treatment can result in favorable efficacy and a good safety profile.

KEYWORDS

intrahepatic cholangiocarcinoma, PD-1 antibody, PD-L1 antibody, immune checkpoint inhibitors, immunotherapy

Introduction

Intrahepatic cholangiocarcinoma (ICC) is a type of adenocarcinoma that originates from the epithelium of the secondary and upper bile duct branches within the liver. The incidence of ICC is second only to that of hepatocellular carcinoma (HCC) (1). It is notable that ICC has a greater tendency to invade and metastasize compared to HCC, leading to significantly shorter OS for ICC patients (2). Surgical resection remains the preferred treatment for ICC patients. However, because early symptoms are often non-specific, many patients do not seek medical attention in time for surgery at the initial diagnosis. Furthermore, ICC is highly malignant, with low rates of surgical resection and a recurrence rate of 60-70% within five years after surgery (3). In recent years, the incidence and mortality rates of ICC have increased globally, with particularly high rates observed in Asian populations compared to those in Europe and North America (4, 5).

The effectiveness of the chemotherapy combination of Gemcitabine and Cisplatin (GP) remains limited (6). In recent years, ICIs have demonstrated promising results in the treatment of various malignancies, including colorectal cancer, non-small cell lung cancer, and malignant melanoma. By inhibiting the protein expression of immunosuppressive checkpoints, ICIs reduce immunosuppression and enhance T-cell activity, ultimately enabling the destruction of cancer cells and the production of an anti-tumor response (7, 8). Among these, inhibitors targeting programmed cell death protein 1 (PD-1) and programmed cell death ligand 1 (PD-L1) are the most extensively utilized. PD-1 is expressed in activated T cells, while PD-L1 is expressed on the surfaces of various immune system cells (9). Moreover, high PD-L1 expression is closely related to low histological differentiation, advanced stage, and poor prognosis of the tumor (10). Therefore, PD-L1 expression can be used as an indicator to assess the malignancy and prognosis of cholangiocarcinoma.

Many patients with advanced ICC have no indication for surgical or biological resection, and the prognosis for these patients is poor. It is our objective to improve their survival rate and extend their lifespans as much as possible. We aim to achieve this through the use of ICIs. However, there is a lack of clinical trial data on the efficacy of these agents in ICC, as well as on the optimal timing for initiating immunotherapy. Whether early ICIs administration can improve outcomes for these patients? Therefore, this study aims to evaluate the efficacy and safety of PD-1 and PD-L1 antibodies in the treatment of advanced ICC.

Materials and methods

1. Study subjects: Review and collect electronic data of patients at the Second Affiliated Hospital of Chongqing Medical University who have received PD-1 and PD-L1 antibody drug therapy for advanced ICC since their admission to the present date of 30 April 2024. Inclusion criteria: ①age≥18 years; ②pathological examination confirmed the diagnosis of ICC; ③Eastern Cooperative Oncology Group (ECOG) score ≤ 2 points; ④stage II to IV ICC patients who had undergone first-line treatment (tumor resection, local treatments such as transcatheter arterial chemoembolization (TACE), radiofrequency ablation (RA), high-intensity focused ultrasound (HIFU) therapy, and particle implantation, radiation therapy, chemotherapy, symptomatic treatment), and there is no evidence to suggest that radical surgical excision is a viable option for surgical or functional resection at present; ⑤Child-Pugh classification standard is A-B; ⑥never used ICIs before. Exclusion criteria: ①combined with other malignant tumors or serious illnesses that may affect a patient's life, such as cardiopulmonary, liver and kidney failure, serious progressive infection; ②previously received anti-tumor immunotherapy; ③those not adhering to a regular medication regimen; ④those with contraindications to the use of ICIs.
2. Treatment procedure: A total of 96 ICC patients were enrolled in the study. Of these, 42 patients received immunotherapy as adjuvant treatment following first-line surgery, local therapy, chemoradiotherapy, etc. (Group1), while 54 patients were treated after ICC progression (Group2). (Figure 1) 48 patients received ICIs as monotherapy, 16 patients received ICIs in combination with Lenvatinib, 30 patients were treated with ICIs alongside chemotherapy, and 2 patients received a regimen combining ICIs, Lenvatinib, and chemotherapy. The PD-1 inhibitors included Sintilimab (200mg/dose), Camrelizumab (200mg/dose), Tislelizumab (200mg/dose), and Toripalimab (200mg/dose). The PD-L1 inhibitors included Durvalumab (1000mg/dose). The drugs are administered intravenously once every three weeks. Patients with hepatitis B virus (HBV) infection receive standard antiviral treatment concurrently with immunotherapy.
3. Observation Endpoints: The primary endpoints of this study are progression-free survival (PFS) and overall survival (OS). PFS is defined as the duration from the initiation of immunotherapy to the documented date of disease progression. OS is defined as the time from the initiation of immunotherapy to the date of death from any cause or the date of the last follow-up. Throughout the treatment and follow-up period, the safety profile is assessed by evaluating adverse events (AEs) in accordance

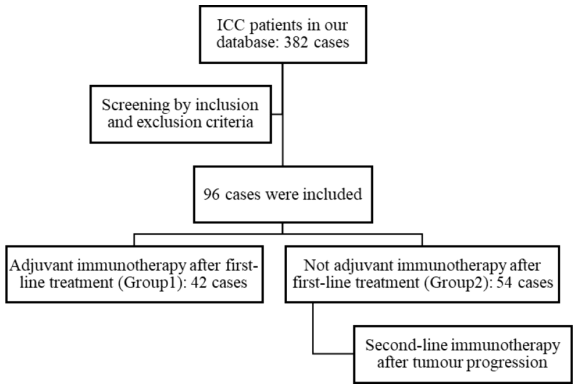

FIGURE 1
Experimental design and grouping flowchart.

with the Common Terminology Criteria for Adverse Events (CTCAE) version 5.0.

4. Data Collection: The clinical information, laboratory test results, and imaging data for the enrolled patients are gathered from the hospital's electronic medical record system. This includes the patient's name, gender, age, ECOG performance status, liver function (The Child-Pugh classification standard), TNM stage, pathological biopsy information, lesion characteristics (tumor diameter, intrahepatic and extrahepatic metastasis, lymph node metastasis, vascular invasion), HBV infection, and medication information.
5. Follow-up: Patients received immunotherapy underwent a complete blood count, liver and kidney function tests every 3 weeks, and abdominal enhanced CT or MRI every 3 months. For patients who were not undergo regular imaging, the observation indicators were the significant increase of tumor markers CA199 and CEA indicating disease progression. Follow-up methods included re-visits, medical record reviews, and telephone follow-ups. The most recent follow-up was conducted on 30 April 2024. For patients with disease progression, the endpoint event is defined as the time of tumor progression, while for patients without disease progression, the endpoint event is defined as the end of the study. Patients lost to follow-up are considered to have the endpoint event at the time of their loss to follow-up.
6. Statistical analysis: The SPSS 25.0 software was employed for the processing and analysis of the data. The baseline data of the two groups of patients were compared using the independent samples t-test and the chi-squared test, with a significance of $P < 0.05$. The Kaplan-Meier method was employed to perform survival analysis and generate survival curves. The statistical significance of differences between groups was evaluated using the log-rank test, with a significance level of $P < 0.05$.

Results

1. General information: This study included a total of 96 patients. The baseline characteristics of the patients are shown in Table 1. The group consisted of 60 males and 36 females. Before using ICIs, 48 patients undergone surgical resection previously; 37 patients received local anti-tumor treatment, including: TACE, RA, HIFU therapy, and particle implantation; 6 patients received radiotherapy; 35 patients received chemotherapy; and 9 patients received symptomatic treatment only. 42 patients were treated with immunotherapy as adjuvant therapy following first-line treatment, while 54 patients were treated following tumor progression. During immunotherapy, 48 patients received single-agent immunotherapy, 16 received combination immunotherapy with Lenvatinib, 30 received combination immunotherapy with chemotherapy, and 2 patients received a triple combination of chemotherapy, immunotherapy and Lenvatinib. Specific medication details are as follows: Sintilimab in 27 cases, Camrelizumab in 27 cases, Toripalimab in 19 cases, Tislelizumab in 18 cases, and Durvalumab in 5 cases.
2. Clinical efficacy: As of 30 April 2024, 85 (88.54%) of the 96 patients experienced disease progression. Among the

TABLE 1 Patient baseline data.

Characteristics	Group1 (42)	Group2 (54)	*P*
Male/Female【case (%)】	27 (64.3)/15 (35.7)	33 (61.1)/21 (38.9)	0.750
age (years)	56 ± 10.529	58 ± 9.793	0.586
ECOG performance status【case (%)】			0.941
0	12 (28.6)	17 (31.5)	
1	24 (57.1)	29 (53.7)	
2	6 (14.3)	8 (14.8)	
Child-Pugh standard【case (%)】			0.384
A (5-6)	32 (76.2)	45 (83.3)	
B (7-9)	10 (23.8)	9 (16.7)	
HBsAg(+)【case (%)】	10 (23.8)	17 (31.5)	0.407
Liver cirrhosis【case (%)】	4 (9.5)	6 (11.1)	0.801
Pathological differentiation degree 【case (%)】			0.864
poorly differentiated	9 (21.4)	14 (25.9)	
moderately to poorly differentiated	8 (19.0)	11 (20.3)	

(Continued)

TABLE 1 Continued

Characteristics	Group1 (42)	Group2 (54)	*P*
moderately differentiated	18 (42.8)	22 (40.7)	
moderately to well differentiated	2 (4.7)	3 (5.5)	
well differentiated	3 (7.1)	1 (1.8)	
unclear	2 (4.7)	3 (5.5)	
number【case (%)】			0.595
single	25 (59.5)	35 (64.8)	
multiple	17 (40.4)	19 (35.1)	
tumor characteristics【case (%)】			
vascular invasion	18 (42.8)	22 (40.7)	0.835
lymph node metastasis	30 (71.4)	31 (57.4)	0.157
intrahepatic and extrahepatic metastasis	21 (50.0)	35 (64.8)	0.203
maximum diameter【case (%)】			0.824
≤2cm	3 (7.1)	5 (9.3)	
2-5cm	17 (40.5)	24 (44.4)	
5-10cm	20 (47.6)	24 (44.4)	
>10cm	2 (4.8)	1 (1.9)	
TNM stage【case (%)】			0.887
II	5 (11.9)	6 (11.1)	
IIIA	4 (9.5)	3 (5.5)	
IIIB	17 (40.4)	22 (40.7)	
IV	16 (38.0)	23 (42.5)	

(gender, age, ECOG performance status, The Child-Pugh classification standard, TNM stage, pathological biopsy information, tumor diameter, intrahepatic and extrahepatic metastasis, lymph node metastasis, vascular invasion, HBV infection).

patients, all 54 patients in Group2 experienced disease progression; Of the 42 patients in Group1, 31 progressed, 7 did not progress, and 4 were lost to follow-up. The median PFS=5.63 months (95%CI: 3.12~8.14) in patients who were initiated immunotherapy immediately after first-line treatment was significantly longer than the PFS=2.5 months (95%CI: 1.83~3.17) in patients who were not (P=0.002) (Figure 2A). Among the 54 patients in Group2, the median PFS following immunotherapy was 4.13 months (95%CI: 0.88-7.38) (Figure 2B). A comparison of the median PFS of two groups of patients initiated immunotherapy in first-line treatment (5.63 months, 95% CI: 3.12~8.14) and those initiated immunotherapy in second-line treatment (4.13 months, 95%CI: 0.88~7.38) revealed no significant difference between the two groups (P=0.406) (Figure 2C). Patients who initiated immunotherapy as adjuvant therapy had too few deaths to estimate OS. The median OS of patients who were treated after disease progression was 27.53 months (95% CI: 13.10~41.96), and the difference in OS between the two groups was not statistically significant (P=0.360) (Figure 2D). Of those 96 patients, 22 died (22.92%), 36 survived, and 38 were lost to follow-up. The causes of death included severe infection or septic shock caused by tumor progression (7 cases), gastrointestinal bleeding (4 cases), liver failure (1 case), multiple systemic metastases (5 cases), cancer cachexia (3 cases), and unknown cause (2cases).

3. Adverse events (AEs) and safety: Among the 96 patients, 18 cases (18.75%) experienced adverse events of varying degrees, with 3 cases (3.13%) experiencing AE grade≥3. Among the cases, 9 cases (9.38%) exhibited mild liver function damage, which was primarily manifested as increased levels of AST or ALT; 3 cases (3.13%) developed immune-related rash, presenting as skin itching. Thyroid dysfunction occurred in 2 cases (2.08%), mainly manifested as hypothyroidism. Hyperthermia occurred in 1 case (1.04%), considered to be associated with tumor progression and abdominal infection. 1 case (1.04%) exhibited immune-related pneumonia; 1 case (1.04%) exhibited newly developed hepatic hemangioma; 1 case (1.04%) exhibited thrombocytopenia, considered to be related to the side effects of chemotherapy drugs. All the above AEs recovered without sequelae after symptomatic treatment.

Discussion

In recent years, immunotherapeutic drugs have brought about significant advances in cancer therapy, with 10% to 20% of ICC patients who were resistant to chemotherapy achieving remission following immunotherapy (11). Current immune-related therapies include ICIs targeting PD-1, PD-L1, and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4). Vaccines, adoptive cell therapy and non-specific immunomodulators are also available. Among these, PD-1 and PD-L1 antibody drugs are the most widely used. However, there are differences between them in terms of mechanism of action, clinical efficacy, adverse reactions, cost, and other factors. In our research, most patients were treated with domestic PD-1 inhibitors, likely influenced by the availability of healthcare coverage for such medications and the financial circumstances of the patients.

Currently, there are many limitations in the study of immunotherapy in cholangiocarcinoma, and comprehensive studies on the optimal timing, indications and efficacy of different drugs are still lacking. Both KEYNOTE-028 and KEYNOTE-158 studies suggest that ICIs have a significant impact on the treatment of advanced cholangiocarcinoma (12). However, most patients are often treated with multiple regimens in the real world, and the efficacy of single-agent immunotherapy in advanced cases remains limited. This study includes various treatment regimens, such as single-agent immunotherapy, doublet therapy, and triplet therapy. A retrospective study from China showed that PD-1 inhibitor combined with Lenvatinib prolonged survival in those with advanced cholangiocarcinoma who had not responded to chemotherapy (13). The results of the follow-up TOPAZ-1 trial indicate that patients with advanced cholangiocarcinoma exhibited a notable improvement in OS, PFS, and ORR when treated with a combination of Durvalumab and the GP regimen and the drug's safety profile was within acceptable range (14). This is the world's first successful Phase III clinical trial to combine chemotherapy with immunotherapy as a first-line treatment for cholangiocarcinoma. Currently, Durvalumab combined GP regimen has been adopted by definitive guidelines and is recommended as a first-line treatment option (15, 16).

In 2023, Zhou Jian's team reported a triple combination regimen of chemotherapy, targeted therapy, and immunotherapy with the monoclonal antibody Toripalimab combined with Lenvatinib and the chemotherapy regimen GEMOX (gemcitabine + oxaliplatin) for the treatment of advanced ICC. The results demonstrated an 80% ORR, with median OS and PFS of 22.5 months and 10.2 months, respectively. These findings represent a significant improvement in efficacy compared to previous monotherapy or combination therapy regimens, with manageable AEs occurring in over half of the patients. This suggests that the treatment of advanced ICC can be gradually extended to chemotherapy, immunotherapy and targeted triple drug (17).

This study included 96 patients with advanced ICC, of whom 85 experienced disease progression. The median PFS of patients who received PD-1 or PD-L1 inhibitors as adjuvant therapy after prior first-line treatment was longer than that of those who did not receive active immunotherapy {5.63 months (95%CI: 3.12~8.14) VS 2.5 months (95%CI: 1.83~3.17)}(P=0.002). This suggests that once diagnosed with ICC, the proactive use of ICIs at an early stage will

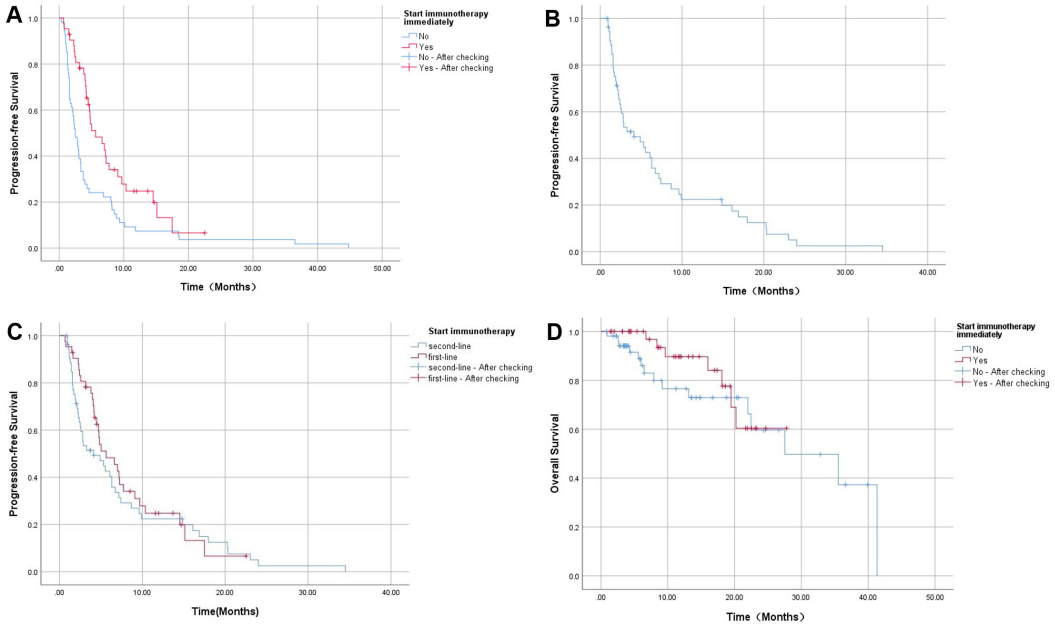

FIGURE 2
(A) The comparison of PFS between the immunotherapy adjunct group and the non-adjunct therapy group; **(B)** PFS of patients receiving immunotherapy after tumor progression; **(C)** The comparison of PFS with immunotherapy in different lines treatment; **(D)** The comparison of OS between the immunotherapy adjunct group and the non-adjunct therapy group.

significantly enhance patient prognosis in comparison to initiating immunotherapy after tumor progression. This indicates that anti-tumor immunotherapy should be introduced as early as possible. For the 54 patients treated after tumor progression, the mPFS after immunotherapy was 4.13 months (95%CI: 0.88~7.38), suggesting that ICIs as a post-line treatment can also appropriately prolong PFS. However, the lack of controlled trials comparing the two groups in second-line treatment introduces some potential bias into the conclusions. Due to the aggressive nature of ICC and its rapid progression, the PFS with immunotherapy in different lines therapy and the final OS between the two groups were not significantly different. In terms of safety, 18 (18.75%) of the 96 patients who experienced AEs recovered following conservative treatment, and there were no deaths related to the treatment. In conclusion, ICIs were well tolerated in ICC therapy and had controlled toxic reactions.

This study still has several limitations. Firstly, it is challenging to rigorously control a single variable in real-world. Some patients received targeted therapies or chemotherapy, which introduces a degree of bias into the efficacy outcomes. Secondly, the study employed a diverse range of drugs, and the sample size was limited, which precluded further stratification of drug types. Most of the advanced ICC patients were in critical condition at the time of treatment, with rapid tumor progression. Additionally, some patients requested to cease treatment, resulting in a significant number of lost follow-ups. The study on PFS of patients undergoing immunotherapy with ICIs after relapse and metastasis is a single-center study lacking a control trial. PD-1 and PD-L1 inhibitors have a potential to significantly extend PFS and appear to have a favorable safety profile for treating advanced ICC patients. Subsequent randomized, large-scale, and prospective trials are essential to optimize the use of immunotherapy in these ICC patients.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving humans were approved by the Ethics Committee of the Second Affiliated Hospital of Chongqing Medical University. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

ZP: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. JD: Conceptualization, Investigation, Methodology, Writing – review & editing. ST: Conceptualization, Investigation, Methodology, Writing – review & editing. JS: Conceptualization, Investigation, Methodology, Writing – review & editing. TS: Conceptualization, Funding acquisition, Investigation, Methodology, Supervision, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Enhanced antitumor activity of combined hepatic arterial infusion chemotherapy with Lenvatinib and PD-1 inhibitors in unresectable hepatocellular carcinoma: a meta-analysis

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Background: Hepatic arterial infusion chemotherapy (HAIC) is increasingly recognized as a primary treatment option for patients with unresectable hepatocellular carcinoma (uHCC), providing a focused treatment for localized tumors. The combination of lenvatinib, a multikinase inhibitor, with PD-1 inhibitors has demonstrated significant survival benefits in HCC. This meta-analysis aims to assess whether the integration of HAIC with lenvatinib and PD-1 inhibitors (referred to as the HAIC-L-P group) leads to better treatment effectiveness and security compared to lenvatinib and PD-1 inhibitors alone (L-P group) in uHCC.

Methods: An exhaustive search of the literature was conducted, including PubMed, the Cochrane Library, Embase, ClinicalTrials.gov, and Web of Science, from the start of each database until September 2024, to ensure a thorough and up-to-date compilation of relevant studies. Extract data on outcome measures such as overall survival (OS), progression-free survival (PFS), objective response rate (ORR), disease control rate (DCR), and adverse events (AEs). Subsequently, meta-analyses were performed using RevMan 5.4 to quantitatively evaluate the aggregated effect of the HAIC-L-P regimen versus the L-P regimen alone.

Results: In our systematic meta-analysis of eight retrospective cohort studies, the HAIC-L-P regimen demonstrated markedly enhanced OS, with an HR of 0.54 (95% CI: 0.45-0.64; $p < 0.00001$), and enhanced 1-year and 2-year OS rates. Superior PFS was also observed in the HAIC-L-P group, with an HR of 0.64 (95% CI: 0.55-0.75; $p < 0.0001$), and higher 1-year and 2-year PFS rates. Response rates were markedly higher in the HAIC-L-P group, with an ORR risk ratio of 2.15 (95% CI: 1.84-2.50; $p < 0.00001$) and a DCR risk ratio of 1.28 (95% CI: 1.20-1.43; $p < 0.0001$). The AEs classified as grade 3 or above were elevated in the HAIC-L-P group, with notable risk ratios for vomiting, elevated AST, elevated ALT, thrombocytopenia, neutropenia, and hyperbilirubinemia. No life-threatening AEs were reported.

Conclusion: The HAIC-L-P regimen correlated with enhanced tumor responses and prolonged survival, alongside manageable adverse effects, indicating its potential as a viable therapeutic strategy for individuals afflicted with uHCC.

Systematic review registration: https://www.crd.york.ac.uk/PROSPERO/, identifier CRD42024594109.

KEYWORDS

hepatic arterial infusion chemotherapy (HAIC), hepatocellular carcinoma (HCC), Lenvatinib, PD-1 inhibitors, meta-analysis, unresectable cancer

Introduction

Hepatocellular carcinoma (HCC) is a prevalent malignancy that accounts for a significant proportion of cancer-related deaths globally (1). For early-stage HCC that is localized within the liver, surgical intervention or ablation remains the treatment of choice (2). Unfortunately, a substantial number of victims present with advanced-stage disease have progressed beyond the scope of resectability. Consequently, there has been a surge of interest in combined therapeutic strategies for unresectable hepatocellular carcinoma (uHCC). Systemic therapy opens new treatment possibilities for uHCC patients, significantly improving patient outcomes (3). For individuals with advanced HCC, lenvatinib, a multi-pathway tyrosine kinase inhibitor, is recommended for systemic therapy, offering satisfactory survival durations and therapeutic efficacy (4, 5). Immunotherapeutic agents, such as PD-1/L1 inhibitors, bolster T-cell activity and exert significant antitumor effects. These agents have demonstrated encouraging therapeutic results for individuals grappling with advanced HCC (6). Beyond systemic therapies, local treatment modalities have demonstrated the capacity to enhance treatment outcomes in patients battling advanced HCC. Hepatic arterial infusion chemotherapy (HAIC) entails the ongoing delivery of chemotherapy drugs via a catheter placed in the hepatic artery, increasing local intrahepatic drug concentrations while reducing systemic toxicity (7). Research has suggested that HAIC is capable of substantially extending the overall survival (OS) for individuals suffering from advanced HCC, and emerges as a viable and secure therapeutic strategy for those with portal vein invasion (8, 9).

A range of combined therapeutic strategies have demonstrated the potential to enhance patient outcomes and possibly transform uHCC into a resectable condition. Considering the unique anti-cancer properties of tyrosine kinase inhibitors (TKIs), PD-1 inhibitors, and hepatic arterial infusion chemotherapy (HAIC), the concurrent use of these three treatment modalities could lead to complementary advantages and show significant potential for securing positive treatment results in advanced HCC patients. In recent clinical investigations, the integration of HAIC with lenvatinib and PD-1 inhibitors has exhibited encouraging outcomes (10, 11). Consequently, our systematic meta-analysis was designed to assess the comparative effectiveness and safety of this three-pronged approach versus the dual regimen of PD-1 inhibitors linked with lenvatinib, to identify more potent therapeutic strategies for uHCC sufferers.

Materials and methods

Adhering to the PRISMA guidelines (12), the article presents outcomes with rigorous transparency and detail. The meta-analysis has been registered with PROSPERO(CRD42024594109).

Search strategy

Our literature retrieval strategy encompassed a systematic search of English-language databases such as PubMed, EMBASE, ClinicalTrials.gov, the Cochrane Library, and the Web of Science. The search terms we utilized spanned a range of relevant medical subject headings (MeSH terms), encompassing hepatocellular carcinoma's various designations like "liver cancer" or "HCC", as well as terms specific to our treatment of interest, including "hepatic arterial infusion chemotherapy" or "HAIC", and the immunotherapeutic agents "PD-1/L1" and " immunological checkpoint inhibitors". Additionally, we incorporated the targeted therapy drug "Lenvatinib" or its trade name "Lenvima" into our search criteria to ensure comprehensive coverage of the topic. The search language is limited to English, and the search continues until September 30, 2024. Two researchers independently searched according to the unified search strategy and the literature was rigorously evaluated and selected based solely on the established criteria for inclusion and exclusion.

Study selection

Inclusion criteria: 1) study design: eligible studies included published randomized controlled trials (RCT) as well as retrospective cohort studies (RCS); 2) patients with hepatocellular carcinoma diagnosed by imaging or pathology and no chance of

surgery; 3) treatment approaches: the experimental cohort received a combination remedy comprising HAIC, Lenvatinib, and a PD-1 inhibitor, whereas the control cohort was administered Lenvatinib in conjunction with a PD-1 inhibitor; 4) principal endpoints: the trial centered on evaluating ORR, DCR, OS, PFS, and the incidence of AEs, with the stipulation that at least one parameter related to survival was mandatory for assessment purposes.

Exclusion criteria: 1) inconsistent intervention measures; 2) observation of inconsistent outcome indicators; 3) articles were medical record reports, meeting abstracts, letters, a Meta-analysis, reviews, animal experiments, and repetitive articles; 4) no control group; 5) research that cannot obtain full text or data cannot be extracted.

Data extraction and quality assessment

Data extraction from eligible studies was conducted independently by two reviewers, adhering to the predefined inclusion-exclusion criteria. To ensure accuracy, a third reviewer performed a cross-check of the extracted data. Any discrepancies that arose between the reviewers were resolved through consensus discussions. The data extracted encompassed several key variables: first author, treatment modalities, publication dates, sample sizes, participant demographics (including gender and age), Child-Pugh classification, ECOG PS, BCLC stage, and post-treatment outcome measures. These outcome measures included ORR, DCR, OS, and PFS. Additionally, AEs were recorded. It is noteworthy that all studies incorporated in this meta-analysis were retrospective. For assessing methodological quality, we employed the Newcastle Ottawa Scale (NOS) (13), providing a standardized framework for evaluating the studies' rigor, with a total score of 9 points, including cohort selection, comparability, exposure, and ≥ 6 points were considered high quality.

Statistical analysis

In this study, we employed RevMan 5.4 software to perform a systematic meta-analysis of the collected data. The hazard ratios (HRs), accompanied by their respective 95% confidence intervals (CIs), served as the primary effect measure for analyzing OS and PFS. For binary variables, including 1-and 2-year OS and PFS, ORR, DCR, and AEs, we employed relative risks (RR) and their corresponding 95% CIs as the effect indicators. Heterogeneity was assessed using the I^2 statistic; a fixed-effects model was used when I^2 was under 50% and the P-value was above 0.1, otherwise, we resorted to employing a random-effects model. Evaluating publication bias through the analysis of funnel plots. A P-value below 0.05 indicates significant disparities.

Result

Ultimately, the study encompassed 8 articles (14–21). A total of 390 eligible articles were retrieved, and 47 duplicates were automatically de-duplicated and manually excluded by EndNote; titles and abstracts were scrutinized to exclude off-topic articles, followed by a full-text review that culminated in the selection of 8 pertinent articles for this meta-analysis, all of which were retrospective cohort studies (RCS). The literature selection process is graphically represented in Figure 1.

Study characteristics and risk of bias

The features of the incorporated studies are summarized in Table 1. A comprehensive analysis encompassing 1174 patients was conducted across 8 studies, with all participants hailing from China between the years 2021 to 2024. Specifically, 654 patients diagnosed with uHCC underwent HAIC-L-P triple therapy, whereas 520 patients received the L-P dual therapy. The NOS was applied to appraise the methodological rigor of the enrolled studies, with each study securing a score of 7 or higher on the NOS, indicating a high standard of quality, as shown in Table 1.

Meta-analysis outcomes

Response outcomes

Eight studies reported on both the ORR and DCR across both groups. These studies exhibited no significant statistical heterogeneity, with $I^2 < 50\%$, prompting the use of a fixed-effects model for our analysis. The findings indicated that, compared to the L-P group, individuals with uHCC in the HAIC-L-P group experienced markedly improved ORR (RR = 2.15; 95% CI: 1.84–2.50; P < 0.00001) and DCR (RR = 1.28; 95% CI: 1.19–1.37; P < 0.00001), as depicted in Figure 2.

Overall survival and progression-free survival

Only four studies reported OS and PFS data for both cohorts, including HRs and 95% CIs. Our analysis showed that the HAIC-L-P treatment notably improved OS (HR = 0.54, 95% CI: 0.45–0.64, P < 0.00001) and PFS (HR = 0.64, 95% CI: 0.55–0.75, P < 0.0001) in individuals with uHCC compared to the L-P regimen. The enhanced therapeutic benefit of HAIC-L-P is depicted in Figure 3.

Among the studies, eight reported 1-year OS and seven reported 2-year OS, while eight and five studies reported 1-year and 2-year PFS, respectively. Subsequent meta-analyses demonstrated that individuals with uHCC in the HAIC-L-P group demonstrated a markedly enhanced 1-year OS (RR = 1.31; 95% CI: 1.17–1.47, P < 0.00001), 2-year OS (RR = 1.67; 95% CI: 1.25–2.24, P = 0.0006), 1-year PFS (RR = 2.33; 95% CI: 1.83–2.97, P < 0.00001), and 2-year PFS (RR = 2.02; 95% CI: 1.18–3.45, P = 0.01) compared to the L-P group. The therapeutic benefit of the HAIC-L-P combination therapy was found to be superior to that of the L-P group, as depicted in Figure 3.

Security assessment

Adverse events

All studies documented varying levels of AEs across both patient groups. Predominantly, the AEs were mild, and there

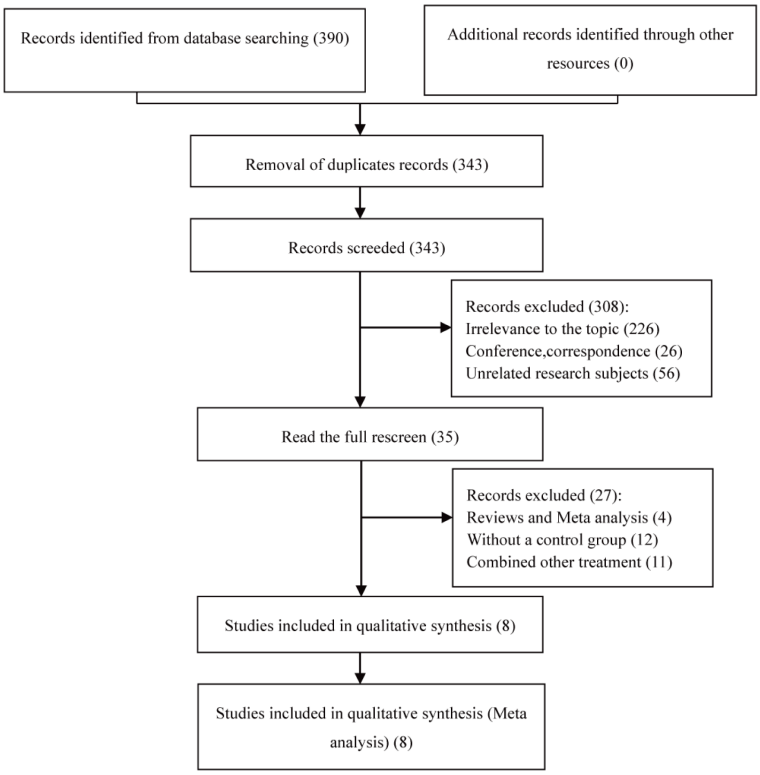

FIGURE 1
Literature screening and selection process.

were also reports of adverse reactions of grade ≥ 3, but there were no deaths. In terms of frequency of all grades AEs, the HAIC-L-P group exhibited a greater likelihood of experiencing Abdominal pain(RR =1.97, 95%CI: 1.27-3.08, P=0.003), Decreased appetite(RR =1.47, 95%CI: 1.21-1.78, P=0.0001), Vomiting(RR =2.60, 95%CI: 1.46-4.60, P=0.001), Elevated AST(RR =2.42, 95%CI: 1.41-4.17, P=0.001), Elevated ALT(RR =2.00, 95%CI: 1.34-2.98, P=0.0007), Thrombocytopenia(RR =2.00, 95%CI: 1.34-2.98, P=0.0007), Anaemia(RR =1.69, 95%CI: 1.23-2.32, P=0.001), Neutropenia(RR =2.80, 95%CI: 1.27-6.18, P=0.01), Hyperbilirubinacemia(RR =1.54, 95%CI: 1.03-2.29, P=0.03) than the L-P group.

Regarding AEs classified as grade 3 or above, the HAIC-L-P group exhibited elevated risk ratios for Vomiting (RR = 3.98, 95% CI: 1.51–10.50, P = 0.005), Elevated AST (RR = 2.67, 95% CI: 1.66–4.28, P = 0.0001), Elevated ALT (RR = 1.85, 95% CI: 1.20–2.84, P = 0.005), Thrombocytopenia (RR = 3.27, 95% CI: 1.81–5.91, P = 0.0001), Neutropenia (RR = 6.69, 95% CI: 2.08–21.52, P = 0.001), and Hyperbilirubinemia (RR = 2.78, 95% CI: 1.47–5.23, P = 0.001) compared to the L-P group, with these differences attaining statistical significance. A detailed overview of these findings is presented in Table 2.

Publication bias

In our analysis, we employed a funnel plot to evaluate the long-term efficacy between the two treatment groups, specifically examining OS at 1-year and 2-year follow-ups, as well as PFS at the same time points. The results of each funnel plot demonstrated that the respective data points from the included studies were all encompassed within the funnel plot and displayed a generally symmetrical distribution (Figure 4). In general, the likelihood of publication bias is minimal, given the symmetrical distribution of scatter points within the inverted funnel plot.

Discussion

Advanced HCC has a poor prognosis and limited treatment options. Sorafenib (22) recommended by guidelines as a first-line treatment, fails to provide durable antitumor effects, leading to drug resistance and tumor recurrence. With the maturation of interventional therapies, HAIC has gained widespread recognition for its efficacy and minimal systemic side effects due to its localized nature and high drug concentration in the liver (23). Previous research has indicated that HAIC has the potential to augment local drug concentrations, subsequently enhancing therapeutic effectiveness, when compared to transarterial chemoembolization (TACE), HAIC, in combination with oxaliplatin and 5-fluorouracil (5-FU), has demonstrated superior ORR and OS (24). HAIC-based combination treatments target the enhancement of survival times in individuals with uHCC. Hepatitis C virus (HCV) is unequivocally recognized as a principal etiology of chronic liver disease, cirrhosis, and HCC. HCV is classified into distinct genotypes and subtypes based on geographical distribution and transmission risk profiles. These genotypes and subtypes are intricately linked to viral load and

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TABLE 1 Basic characteristics and quality evaluation.

Study	Country	Design	Group/ Participants	Sex (M/F)	Age(years)	Child-Pugh class (A/B)	ECOG PS (0-1/2)	BLCL (B/C)	NOS score
Xu 2024 (14)	China	RCS	HAIC-L-P /103	91/12	52.0±8.82	84/19	96/7	4/99	7
			L-P /61	54/7	56.0±7.88	50/11	58/3	2/59	
Li rx 2024 (15)	China	RCS	HAIC-L-P /81	69/12	≥60:68 <60:13	/	/	9/109	8
			L-P /81	68/13	≥60:72 <60:9	/	/	13/101	
Diao lf 2023 (16)	China	RCS	HAIC-L-P /58	49/9	≤ 50:16 > 50:52	49/9	27/31	24/34	8
			L-P /63	50/13	≤ 50:5 > 50:57	55/8	23/40	25/38	
Guan rg 2024 (17)	China	RCS	HAIC-L-P /127	107/20	52.9±10.88	104/23	/	/	7
			L-P /103	94/9	54.01±11.54	84/19	/	/	
Chen 2021 (18)	China	RCS	HAIC-L-P /84	72/12	52±6.25	71/13	84/0	22 62	8
			L-P /86	71/15	53±6.5	75/11	86/0	21/65	
Fu yz 2023 (19)	China	RCS	HAIC-L-P /89	83/6	/	/	/	/	7
			L-P /53	50/3	/	/	/	/	
Mei 2021 (20)	China	RCS	HAIC-L-P /45	38/7	49.1±10.6	44/1	/	5/40	8
			L-P /25	18/7	49.1±10.6	22/3	/	3/22	
Lou yd 2024 (21)	China	RCS	HAIC-L-P /67	61/6	56.15 ± 7.78	56/11	/	/	8
			L-P /48	44/4	53.48 ± 10.39	37/11	/	/	

M, male; F, female; HAIC,Hepatic arterial infusion chemotherapy; L, lenvatinib; P, PD-1 inhibitors; ECOG, Eastern Cooperative Oncology Group; BCLC, Barcelona Clinic Liver Cancer. NOS, the Newcastle–Ottawa Scale; RCS, retrospective cohort study; /, not reported.

are significant determinants of therapeutic efficacy and disease progression (25). In regions where HCV is endemic, HAIC emerges as a potential first-line treatment option, particularly for patients with advanced HCC characterized by microvascular invasion in the absence of extrahepatic metastasis. Moreover, the combination of local regional therapy with systemic targeted and immunotherapies has garnered significant attention in the treatment of uHCC, and a burgeoning corpus of experimental data validates the potency of the HAIC-lenvatinib-PD-1 axis in combating advanced HCC (26). Yet, a cohesive appraisal of this trimodal therapy's efficacy and safety remains uncharted. Leveraging clinical trial evidence, our study delivers a holistic assessment of this integrated therapeutic approach for uHCC, thereby anchoring clinical practice in a robust scientific framework.

Our meta-analysis reveals that the HAIC-L-P group demonstrated superior outcomes over the L-P group, with extended OS and PFS, along with improved ORR and DCR. The heterogeneity among studies was minimal. We underscore the importance of scrutinizing the principal determinants of heterogeneity when interpreting clinical data and study methodologies. It is recommended that forthcoming research endeavors delve into these determinants to enhance the precision of treatment effect estimations. In this study's indicators (ORR, DCR, OS, 1-year OS, 2-year OS, PFS, 1-year PFS, 2-year PFS), we assessed heterogeneity using I^2 (values <50%) indicating low heterogeneity, leading to the selection of the fixed-effects model. AEs associated with treatment were manageable and did not result in any mortalities. These results align with previous studies. While the L-P combination is an important treatment option for uHCC, its limitations in advanced disease highlight the need for enhanced therapeutic strategies. A recent investigation suggests the L-P group has demonstrated significant antitumor activity in patients with advanced uHCC, however, the HAIC-L-P triple therapy, employed as the initial treatment for HCC patients exhibiting macrovascular invasion, achieves superior ORR and DCR, furthermore, a greater number of patients became eligible for conversion surgery, and the adverse events were deemed tolerable (27). In addition, a recent clinical trial has demonstrated that the HAIC-L-P combination significantly enhances oncological responses and extends survival, thereby offering a more favorable survival prognosis for HCC patients who are unresponsive to TACE (28).

This improvement in patient prognosis with triple therapy is attributed to several mechanisms. On the one hand, HAIC delivers chemotherapeutic agents into the tumor-feeding arteries, inducing cytotoxic effects and creating an ischemic hypoxic environment that leads to tumor necrosis and promotes the production and release of tumor-specific antigens. Inhibitors of the PD-1 pathway may facilitate the generation of tumor antigen-specific memory T cells, thereby amplifying and perpetuating the patient's immune response

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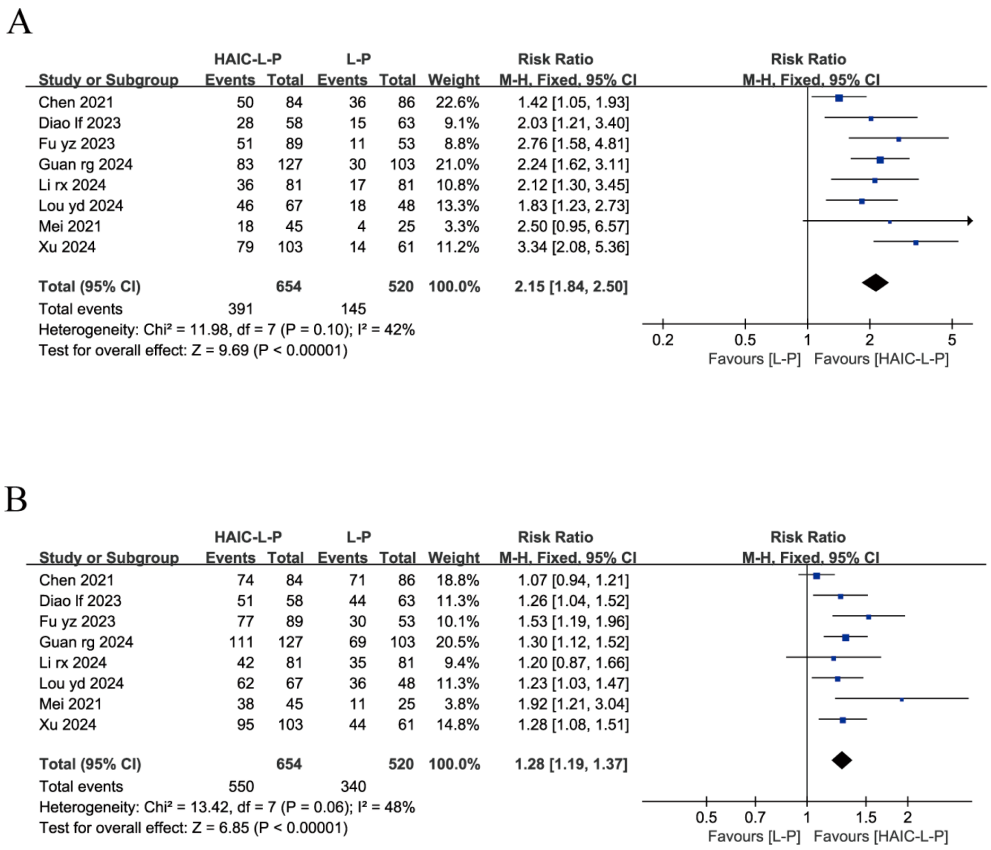

FIGURE 2
Fixed effect model of ORR **(A)** and DCR **(B)** for uHCC with HAIC-L-P vs L-P. ORR, objective response rate; DCR, disease control rate; uHCC, unresectable hepatocellular carcinoma; HAIC, Hepatic arterial infusion chemotherapy; L, lenvatinib; P, PD-1 inhibitors; CI, confidence intervals.

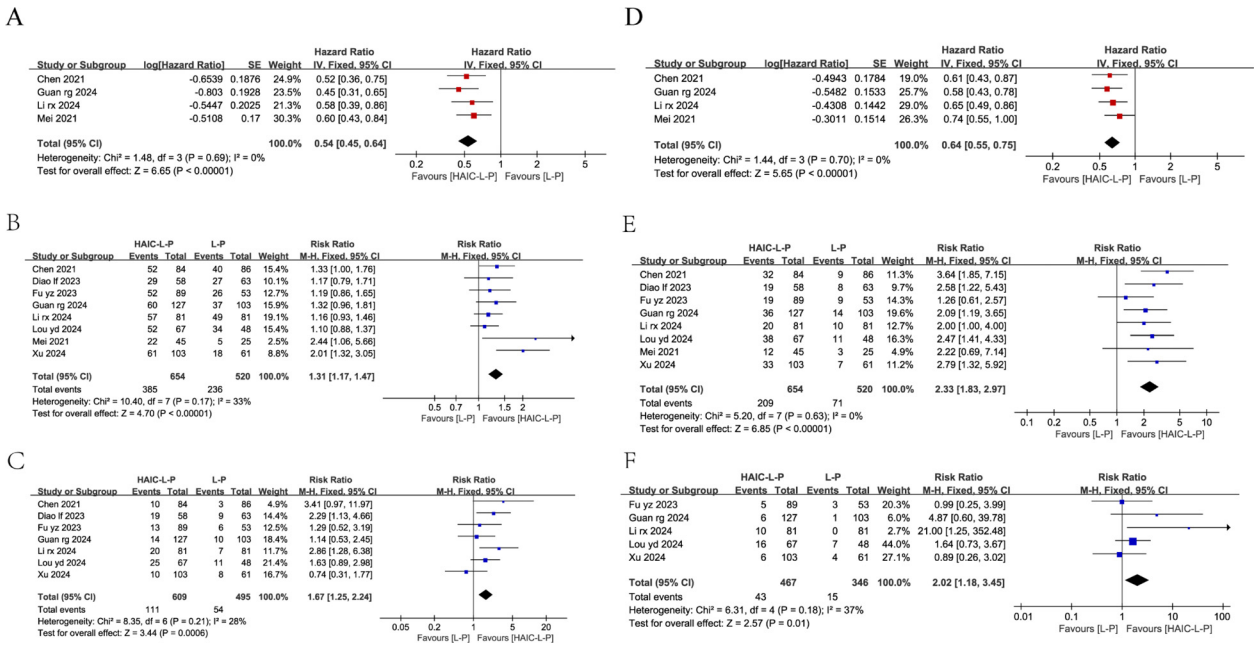

FIGURE 3
Fixed effect model of OS **(A)**, 1-year OS **(B)**, 2-year OS **(C)**, PFS **(D)**, 1-year PFS **(E)**, 2-year PFS **(F)** for uHCC with HAIC-L-P vs L-P. OS, overall survival; PFS, progression-free survival; uHCC, unresectable hepatocellular carcinoma; HAIC, Hepatic arterial infusion chemotherapy; L, lenvatinib; P, PD-1 inhibitors; CI, confidence intervals.

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TABLE 2 Adverse events of HAIC-L-P group vs L-P group.

Adverse Events	All grades			Grade 3-4		
	Studies	RR [95% CI]	P	Studies	RR [95% CI]	P
Diarrhea	8	0.86 [0.66, 1.12]	0.26	5	0.86 [0.37, 2.02]	0.73
Abdominal pain	6	1.97 [1.27, 3.08]	**0.003**	4	2.18 [0.90, 5.29]	0.08
Decreased appetite	7	1.47 [1.21, 1.78]	**0.0001**	3	2.15 [0.99, 4.67]	0.05
Hypertension	6	1.02 [0.87, 1.20]	0.76	4	1.03[0.58, 1.84]	0.92
Vomiting	6	2.60 [1.46, 4.60]	**0.001**	5	3.98[1.51, 10.50]	**0.005**
Rash	7	1.02 [0.75, 1.37]	0.92	4	0.18[0.49, 1.69]	0.77
Hand-foot syndrome	2	0.75 [0.21, 2.72]	0.66	2	2.11[0.61, 7.24]	0.24
Fatigue	8	1.19 [0.99, 1.43]	0.07	4	0.87[0.42, 1.81]	0.71
Elevated AST	5	2.42 [1.41, 4.17]	**0.001**	5	2.67[1.66, 4.28]	**0.0001**
Elevated ALT	7	2.00 [1.34, 2.98]	**0.0007**	6	1.85[1.20, 2.84]	**0.005**
Thrombocytopenia	7	2.63[1.24, 5.58]	**0.01**	6	3.27[1.81, 5.91]	**0.0001**
Hypothyroidism	8	1.06[0.81, 1.38]	0.67	4	1.50[0.66, 3.43]	0.33
Anaemia	4	1.69[1.23, 2.32]	**0.001**	3	2.73 [0.46, 16.02]	0.27
Neutropenia	6	2.80[1.27, 6.18]	**0.01**	4	6.69 [2.08, 21.52]	**0.001**
Proteinuria	5	1.26[0.90, 1.75]	0.18	4	1.41[0.58, 3.38]	0.45
Hyperbilirubinacemia	7	1.54[1.03, 2.29]	**0.03**	5	2.78[1.47, 5.23]	**0.002**

AST, aspartate aminotransferase; ALT, alanine aminotransferase; RR, relative risk; CI, confidence intervals. The bold values indicate that compared to the L-P group, the HAIC-L-P group of uHCC patients experienced more frequent adverse events, which should be taken seriously.

The bold values indicate that compared to the L-P group, the HAIC-L-P group of uHCC patients experienced more frequent adverse events.

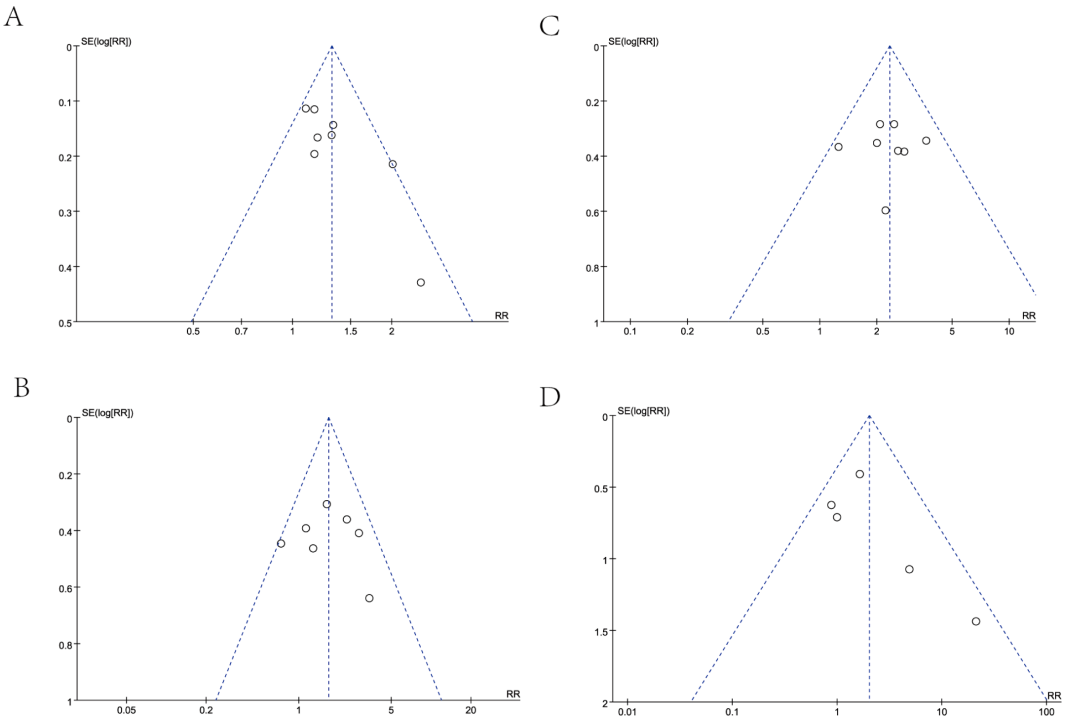

FIGURE 4
funnel plot of 1-year OS **(A)**, 2-year OS **(B)**, 1-year PFS **(C)**, and 2-year PFS **(D)**.

against the tumor (29), HAIC induces ischemic necrosis of tumor tissue while releasing a substantial amount of tumor-specific antigens, thereby enhancing the antitumor immune effects of PD-1 inhibitors (30). On the other hand, lenvatinib, a multi-kinase inhibitor known for its anti-angiogenic properties, can neutralize the vascularization triggered by hypoxia following HAIC. It normalizes blood vessels, fine-tunes the immune contexture of the tumor, and fosters the penetration of immune cells into the tumor mass, lenvatinib exhibits inhibitory effects on IFN-γ signal transduction in tumor cells through targeted modulation of FGFR, thereby amplifying the immunological effects of PD-1 inhibitors against HCC (31, 32). The combined use of PD-1 and lenvatinib can also reverse the immunosuppressive state within the tumor microenvironment, thereby increasing the immune response rate to PD-1 inhibitors (33). The deployment of the triple therapy can establish a robust positive feedback mechanism against tumors, engendering profound and enduring therapeutic responses through a multitude of synergistic pathways.

This study appraised the safety profile of the integrated therapeutic approach, including HAIC, for uHCC patient treatment. The findings indicate that combination therapy, while enhancing therapeutic efficacy, also results in the occurrence of inevitable adverse reactions. The HAIC-L-P group has a higher risk of grade≥3 AEs, including vomiting, bone marrow suppression (thrombocytopenia, neutropenia), and liver function damage (elevated AST, elevated ALT, hyperbilirubinemia). Therefore, special attention should be paid to repeated blood tests for routine, liver function, and if thrombocytopenia, neutropenia, and liver function abnormalities occur, timely treatment with recombinant human interleukin 11, granulocyte colony-stimulating factor injection, and hepatoprotective drugs should be administered. If necessary, the infusion of chemotherapeutic agents should be slowed down or paused. If long-term recurrent vomiting occurs after HAIC treatment, rehydration should be intensified to maintain water-electrolyte balance and intravenous nutritional support should be given appropriately. Other adverse reactions should also be vigilantly monitored for timely treatment. For uHCC patients, although the HAIC-L-P triple therapy may cause more adverse reactions, these can be managed by extending hospital stays and symptomatic treatment, indicating that these reactions are controllable. Consequently, in clinical settings, vigilant attention must be given to AEs induced by the therapeutic regimen to guarantee the safety of treatment administration.

This meta-analysis has some limitations. Firstly, it's crucial to recognize that this meta-analysis, like all others in the field, grapples with inherent variability across the encompassed trials due to differences in baseline patient demographics, disease classification, and therapeutic approaches. Secondly, considering that this study is rooted in a retrospective cohort design and the small number of studies, which may introduce selection bias, publication bias, and heterogeneity concerns, our findings necessitate further validation through more prospective, randomized controlled trials. These trials will furnish us with more robust evidence to ascertain the efficacy of the HAIC-L-P regimen in the treatment of uHCC. This methodological shortfall could inadvertently lead to an overestimation or underestimation of the reported treatment effect sizes. Owing to the scarcity of original studies and available extractable data, this study could not perform further subgroup analyses. Given the Chinese origin of all published studies, the majority of HCC cases in China are primarily associated with hepatitis B virus infection, while in other regions, HCV is the main culprit. Due to variations in healthcare infrastructure, resource availability, and cultural practices, the treatment approaches for viral hepatitis differ across regions, potentially impacting the universality of our research findings, the applicability of these findings to Western populations might be subject to caveats. In our study, the benefits of HAIC-L-P triple therapy may be related to the etiology of the disease. Therefore, we recommend that future analyses should aim to include studies from diverse geographical areas to enhance the global applicability of the research results. By conducting or incorporating international studies in future meta-analyses, a more comprehensive understanding of the efficacy of HCC treatments across different populations can be achieved.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Author contributions

LZ: Formal analysis, Methodology, Writing – original draft. CX: Formal analysis, Software, Writing – review & editing. JD: Data curation, Writing – original draft. YN: Data curation, Writing – original draft.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Establishment of a nomogram based on Lasso Cox regression for albumin combined with systemic immune-inflammation index score to predict prognosis in advanced pancreatic carcinoma

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Purpose: The study aims to establish a nomogram to predict advanced pancreatic carcinoma patients' overall survival (OS), incorporating albumin combined with systemic immune-inflammation index (A-SII) score and clinical characteristics.

Methods: A retrospective study analyzed the clinical data of 205 advanced pancreatic carcinoma patients without antitumor treatment from the Yancheng No.1 People's Hospital between October 2011 and June 2023, and the study divided patients into the training set and the validation set randomly at the proportion of three to one. The A-SII score was divided into scores of 0, 1, and 2 according to the different levels of albumin and SII. Receiver operating characteristic (ROC) curves and time-dependent area under the curve were used to evaluate the predictive ability of the A-SII score. The nomogram1 and nomogram2 were established by the multivariate Cox regression and Lasso Cox regression respectively. The study evaluated the discriminability of nomogram1 and nomogram2 based on C-index and ROC curves to obtain the optimal model. Subsequently, we plotted decision curve analyses (DCA) and calibration curves to estimate the clinical benefit and accuracy of nomogram2.

Results: Lasso Cox regression showed that A-SII score, number of organ metastases, tumor size, chemotherapy, targeted therapy, Neutrophil-to-albumin ratio, and lactate dehydrogenase were independent prognostic factors for the OS of advanced pancreatic carcinoma patients. The C-index and ROC curve of the nomogram2 are better than the nomogram1. Subsequently, the DCA and calibration curve of the nomogram2 demonstrate excellent performance.

Conclusion: The nomogram based on the A-SII score and other independent prognostic factors determined by Lasso Cox regression can accurately predict the OS of patients suffering from advanced pancreatic carcinoma.

KEYWORDS

advanced pancreatic carcinoma, LASSO Cox regression, A-SII score, nomogram, overall survival

1 Introduction

Pancreatic carcinoma (PC) is an extremely aggressive and fatal malignancy with rapidly rising morbidity and mortality. It ranks as the third cause of cancer-related mortality globally, and its five-year overall survival (OS) probability is just 10% (1–4). In order to improve the OS of patients with advanced pancreatic carcinoma, Multi-disciplinary Treatment and Holistic Integrated Medicine have gradually emerged (5, 6), which aim for early discovery, early diagnosis, and early treatment of PC. However, due to the non-specific symptoms of the early stages of pancreatic carcinoma, most patients were already diagnosed with locally advanced or metastatic pancreatic carcinoma when they were detected, and the overall therapeutic effect of advanced pancreatic carcinoma is not obvious (7, 8). Recently, in addition to chemotherapy, other adjuvant treatments such as targeted therapy and immunotherapy have been gradually applied for advanced pancreatic carcinoma (9, 10). However, the prognoses of different patients with the same therapy methods are quite different, which makes the clinical evaluation of prognosis face challenges (11, 12). Therefore, the study aims to find simple and individualized biomarkers that effectively evaluate patients' prognoses and guide clinical decisions.

At present, the tumor–node–metastasis (TNM) classification is identified as the optimal staging system for PC. However, patients with the same substage of advanced pancreatic carcinoma show different prognoses due to their heterogeneity (13). In clinical diagnosis and treatment, clinicians commonly rely on tumor imaging features to assess patient prognosis. For patients with advanced pancreatic carcinoma who are unable to undergo surgery, the inability to evaluate regional lymph node metastases makes specific N stages difficult to be determined accurately (14, 15). Therefore, it is urgent to find new biological markers to predict the survival probability of advanced pancreatic carcinoma. Recently, studies have found that some serum markers reflecting the body's immune inflammation and nutritional status can predict the prognosis of PC, such as neutrophil-to-lymphocyte ratio (NLR), systemic immune-inflammation index (SII), albumin and prognostic nutrition index (PNI), and so on (16–18). However, these markers are single and can't comprehensively predict the prognosis of PC. In addition, there are rarely studies that investigated the relationship between albumin combined with SII (A-SII) score and prognosis of advanced pancreatic carcinoma.

Therefore, the study aims to explore whether the A-SII score and clinical characteristics could accurately predict the survival probability of advanced pancreatic carcinoma. Meanwhile, to eliminate the multicollinearity between different indicators (19), a nomogram will be constructed based on Lasso Cox regression to guide clinical decisions.

2 Materials and methods

2.1 Patients selected

The study included 205 advanced pancreatic carcinoma patients without antitumor treatment admitted to the Affiliated Yancheng No.1 People's Hospital of Nanjing University between October 2011 and June 2023. In this study, advanced pancreatic carcinoma was diagnosed according to the diagnostic criteria of NCCN Clinical Practice Guidelines in Oncology. Inclusion criteria (1): pancreatic carcinoma was diagnosed by cytological biopsy and locally advanced pancreatic carcinoma was diagnosed according to clinical imaging data, or metastatic pancreatic carcinoma (mPC) was confirmed by pathology, (2) age >18 years old, (3) no previous antitumor treatment. Exclusion criteria: (1) patients with other malignant tumors(n=4); (2) patients with incomplete clinical data(n=12); (3) patients lost to follow-up (n=18). According to the above criteria, a number of 205 patients were enrolled in our research (Supplementary Figure S1). Since this study was retrospective and just analyzed the clinical data of the included patients and no human specimens were involved, the informed consent form was waived. This study has been approved by the Ethics Committee of the Affiliated Yancheng No.1 People's Hospital of Nanjing University (approval number: 2024-K-001).

2.2 Data elements

The study collected general information and clinical features of the enrolled patients through querying the Hospital Information System. Objective data, including medical records, imaging findings, and laboratory test results, were used as data sources to reduce the influence of subjective factors. Moreover, the data were collected by two designated researchers following strict inclusion and exclusion criteria within the same time period to reduce

information bias. General information: age, sex, diabetes; Clinical features: (1) tumor information: liver metastases and other metastases, number of organ metastases, primary site and tumor size; (2) Treatment: radiation, chemotherapy, immunotherapy, targeted therapy; (3) Serological indicators: neutrophil count, lymphocyte count, platelet count, serum albumin, lactate dehydrogenase (LDH), carbohydrate antigen 19-9 (CA19-9). All serological indicators were obtained from venous blood collected with an empty belly within 7 days before the first diagnosis of locally advanced or metastatic pancreatic carcinoma.

2.3 Definitions of neutrophil-to-albumin ratio, systemic immune-inflammation index, and A-SII score

Neutrophil-to-albumin ratio (NAR) and SII were calculated according to the following formula: NAR = neutrophil count($\times10^9$/L)/albumin (g/L), SII = platelet count ($\times10^9$/L) × neutrophil count ($\times10^9$/L)/lymphocyte count ($\times10^9$/L). In this study, albumin and SII were combined to establish the A-SII score. The optimal cutoff values for albumin and SII were identified through X-tile. According to the best cutoff values of albumin and SII, the A-SII score was divided into three groups, and the specific scoring rules were as follows: the A-SII score of 0 (high albumin and low SII); The A-SII score of 1 (high albumin and high SII or low albumin and low SII); The A-SII score of 2 (low albumin and high SII). Additionally, we evaluated the predictive ability of the A-SII score using receiver operating characteristic (ROC) curves and time-dependent area under the curve (t-AUC) analyses.

2.4 Follow-up

We used telephone, text messages, or outpatient reviews to follow up with all enrolled patients, and all patients were followed up until death or December 31, 2023. OS was defined as the time from diagnosis of advanced pancreatic carcinoma to death or the time from diagnosis of advanced pancreatic carcinoma to the end of follow-up. The enrolled patients' median follow-up time was 677 days (468-886 days).

2.5 Statistical analysis

In our study, we used the median as the cutoff value of age, and the optimal cutoff values of other continuous variables were calculated by the X-tile software, which fully considered both the survival time and survival status of patients. Data were analyzed with the use of IBM SPSS Statistics (27.0.1) and R software (4.3.3). The included patients were divided into the training set (n=154) and the validation set (n=51) at random according to the proportion of three to one by R software, each variable between the training set and the validation set had no statistical difference ($p > 0.05$). A nomogram was developed using the training set, and its predictive performance was validated using the validation set data to reduce selection bias. The study used the chi-square test or Fisher exact test to evaluate the relationship between the A-SII score and clinical data.

The Univariate and multivariate Cox regression were used to confirm the prognostic factors of advanced pancreatic carcinoma. $P < 0.05$ was considered a statistical difference. We used Lasso regression to eliminate the influence of multicollinearity among the factors with $P < 0.05$ based on the univariate Cox regression. R software was used to establish nomogram1 based on the multivariate Cox regression and nomogram2 based on the Lasso Cox regression. In order to confirm the better nomogram, the study used C-index and AUC to compare the discrimination of the two nomograms. The decision curve analyses (DCA) and calibration curve were used to evaluate the clinical benefit and accuracy of the better nomogram. Finally, the enrolled patients were divided into low risk group and high risk group by the median of total points calculated through the better nomogram. The Kaplan-Meier (K-M) survival difference analysis was performed by the log-rank test. In this study, we considered that $P<0.05$ has a statistical difference.

3 Results

3.1 Patient characteristics

A number of 205 patients with advanced pancreatic carcinoma without antitumor therapy were admitted to our study, and all patients were divided into the training set (n=154) and validation set (n=51) at random according to the proportion of three to one. The median age of the study population was 67 years (38-95 years). This study contained 133 males (64.9%) and 72 females (35.1%). In addition, 77 patients (37.6%) had diabetes history in the study. Otherwise, the majority of patients had liver metastases (69.3%). In terms of adjuvant therapy, the largest number of patients were chemotherapy (58.5%). The rest patients did not receive chemotherapy, potentially due to factors such as advanced age, weakened physical condition, severe cancer pain, or low willingness to undergo treatment. More clinical features and treatment regimens of patients can be found in Table 1.

3.2 A-SII score establishment, assessment, and relationship to clinical data

As shown in Supplementary Figure S2, the optimal cutoff value determined by the X-tile software for albumin was 36.8(g/L), and for SII was 930.9($\times10^9$/L). Univariate and multivariate Cox regression analyses identified albumin and SII as independent prognostic factors in advanced pancreatic carcinoma ($P < 0.05$, Supplementary Table S1). Therefore, we combined albumin and SII to establish the A-SII score. The 12-month ROC curves of the A-SII score indicated that its AUC was 0.741, which was superior to 0.684 for albumin and 0.651 for SII (Supplementary Figure S3A). Furthermore, the t-AUC curves indicated that the A-SII score exhibited superior predictive performance compared to albumin or SII individually (Supplementary Figure S3B).

TABLE 1 Comparison of clinical data between the training set and validation set.

Characteristics	Total (n=205)	Training set (n=154)	Validation set (n=51)	P value
Age, *n* (%)				0.906
≤67 years	112 (54.6)	85 (55.2)	27 (52.9)	
>67 years	93 (45.4)	69 (44.8)	24 (47.1)	
Sex, *n* (%)				1
Male	133 (64.9)	100 (64.9)	33 (64.7)	
Female	72 (35.1)	54 (35.1)	18 (35.3)	
Diabetes, *n* (%)				1
No	128 (62.4)	96 (62.3)	32 (62.7)	
Yes	77 (37.6)	58 (37.7)	19 (37.3)	
Liver metastases, *n* (%)				0.681
No	63 (30.7)	49 (31.8)	14 (27.5)	
Yes	142 (69.3)	105 (68.2)	37 (72.5)	
Other metastases, *n* (%)				0.469
No	130 (63.4)	95 (61.7)	35 (68.6)	
Yes	75 (26.6)	59 (38.3)	16 (31.4)	
Number of organ metastases, *n* (%)				0.227
0	27 (13.2)	22 (14.3)	5 (9.8)	
1	128 (62.4)	91 (59.1)	37 (72.5)	
≥2	50 (24.4)	41 (26.6)	9 (17.6)	
Primary site, *n* (%)				0.05
Head of pancreas	63 (30.7)	42 (27.3)	21 (41.2)	
Neck of pancreas	8 (3.9)	7 (4.5)	1 (2.0)	
Body of pancreas	33 (16.1)	27 (17.5)	6 (11.8)	
Tail of pancreas	44 (21.5)	29 (18.8)	15 (29.4)	
Overlapping lesion of pancreas	57 (27.8)	49 (31.8)	8 (15.7)	
Tumor size, *n* (%)				0.594
≤52 mm	153 (74.6)	113 (73.4)	40 (78.4)	
>52 mm	52 (25.4)	41 (26.6)	11 (21.6)	
Chemotherapy, *n* (%)				0.832
No	85 (41.5)	65 (42.2)	20 (39.2)	
Yes	120 (58.5)	89 (57.8)	31 (60.8)	
Radiation, *n* (%)				0.69
No	182 (88.8)	138 (89.6)	44 (86.3)	
Yes	23 (11.2)	16 (10.4)	7 (13.7)	
Immunotherapy, *n* (%)				0.802
No	177 (86.3)	134 (87.0)	43 (84.3)	

(Continued)

TABLE 1 Continued

Characteristics	Total (n=205)	Training set (n=154)	Validation set (n=51)	P value
Immunotherapy, *n* (%)				0.802
Yes	28 (13.7)	20 (13.0)	8 (15.7)	
Targeted therapy, *n* (%)				0.917
No	178 (86.8)	133 (86.4)	45 (88.2)	
Yes	27 (13.2)	21 (13.6)	6 (11.8)	
LDH, *n* (%)				0.18
≤264 U/L	142 (69.3)	111 (72.1)	31 (60.8)	
>264 U/L	63 (30.7)	43 (27.9)	20 (39.2)	
CA19-9, *n* (%)				0.255
≤10.5 U/mL	23 (11.2)	20 (13.0)	3 (5.9)	
>10.5 U/mL	182 (88.8)	134 (87.0)	48 (94.1)	
NAR, *n* (%)				0.708
≤0.10	87 (42.4)	67 (43.5)	20 (39.2)	
>0.10	118 (57.6)	87 (56.5)	31 (60.8)	
A-SII score, *n* (%)				0.152
0	95 (46.3)	74 (48.1)	21 (41.2)	
1	69 (33.7)	54 (35.1)	15 (29.4)	
2	41 (20.0)	26 (16.9)	15 (29.4)	

As shown in Table 2, there were 95 patients (46.3%), 69 patients (33.7%), and 41 patients (20.0%) with A-SII scores of 0, 1, and 2 respectively. In addition, the A-SII score was significantly relevant with age (P=0.048), primary site (P=0.024), tumor size (P=0.026), LDH (P=0.001), and NAR (P < 0.001). However, the A-SII score had no significant correlations with sex, diabetes, and other markers (P > 0.05).

3.3 Independent prognostic factors for advanced pancreatic carcinoma

As shown in Table 3, 16 variables were subjected to univariate Cox regression, and the results showed that age, other metastases, number of organ metastases, tumor size, chemotherapy, targeted therapy, LDH, NAR, A-SII score had significant correlation with the prognosis of the patients with advanced pancreatic carcinoma (P <0.05). Then, variables with statistical differences in the univariate Cox regression were admitted into the multivariate Cox regression. The final results showed that the number of organ metastases, tumor size, chemotherapy, targeted therapy, NAR, and A-SII score were independent prognostic factors for OS.

Meanwhile, considering the possible collinearity relationship between the variables, we used Lasso regression to analyze the variables with statistical differences in the univariate Cox regression, and the variation characteristics of the coefficients of each variable are shown in Figure 1A. By the cross-validation method, 7 variables were selected at one standard error criteria of minimum which was the optimal penalty coefficient, including the number of organ metastases, tumor size, chemotherapy, targeted therapy, NAR, LDH, and A-SII score confirmed as the independent prognostic factors for OS (Figure 1B).

3.4 Nomogram establishment and validation

Firstly, we established nomogram1 to predict the 3-, 6-, and 12-month OS based on the univariate and multivariate Cox regression (Figure 2A), and meanwhile, nomogram2 was constructed to predict the 3-, 6-, and 12-month OS based on the Lasso Cox regression (Figure 2B). The C-index of the multivariate Cox regression was 0.728 (95%CI: 0.674-0.763) in the training set and 0.779(95%CI: 0.684-0.822) in the validation set. The C-index of the Lasso Cox regression was 0.735 (95%CI: 0.673-0.767) in the training set and 0.791(95%CI: 0.719-0.831) in the validation set. It is clear that the C-index of nomogram2 is superior to nomoram1. Then, the ROC curves of the two nomograms were plotted, and the AUC of nomogram1 in all enrolled patients for predicting 3-, 6-, and 12-month OS respectively reached

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TABLE 2 Relationships between A-SII score and clinical data (n=205).

Factors	A-SII score			X^2	P value
	0(n=95)	1(n=69)	2(n=41)		
Age, *n*				6.08	**0.048**
≤67 years	60	35	17		
>67 years	35	34	24		
Sex, *n*				4.801	0.091
Male	68	38	27		
Female	27	31	14		
Diabetes, *n*				1.027	0.599
No	62	43	23		
Yes	33	26	18		
Liver metastases, *n*				0.368	0.832
No	30	22	11		
Yes	65	47	30		
Other metastases, *n*				2.165	0.339
No	65	42	23		
Yes	30	27	18		
Number of organ metastases, *n*				5.787	0.216
0	13	11	3		
1	65	38	25		
≥2	17	20	13		
Primary site, *n*				17.593	**0.024**
Head of pancreas	28	23	12		
Neck of pancreas	5	3	0		
Body of pancreas	17	9	7		
Tail of pancreas	17	10	17		
Overlapping lesion of pancreas	28	24	5		
Tumor size, *n*				7.308	**0.026**
≤52 mm	79	48	26		
>52 mm	16	21	15		
Chemotherapy, *n*				3.781	0.151
No	34	29	22		
Yes	61	40	19		
Radiation, *n*				2.41	0.3
No	84	59	39		
Yes	11	10	2		
Immunotherapy, *n*				5.902	0.052
No	77	65	35		

(Continued)

TABLE 2 Continued

Factors	A-SII score			X^2	P value
	0(n=95)	1(n=69)	2(n=41)		
Immunotherapy, *n*				5.902	0.052
Yes	18	4	6		
Targeted therapy, *n*				2.09	0.352
No	79	62	37		
Yes	16	7	4		
LDH, *n*				13.444	**0.001**
≤264 U/L	76	46	20		
>264 U/L	19	23	21		
CA19-9, *n*				0.606	0.739
≤10.5 U/mL	10	7	6		
>10.5 U/mL	85	62	35		
NAR, *n*				42.204	**<0.001**
≤0.10	59	27	1		
>0.10	36	42	40		

Statistical differences are indicated in bold.

TABLE 3 Univariate and multivariate Cox regression analyses of all variables for overall survival of advanced pancreatic carcinoma patients.

Variables	Univariate analysis		Multivariate analysis	
	Hazard Ratio(95%CI)	P value	Hazard Ratio(95%CI)	P value
Age				
≤67 years	Reference			
>67 years	1.442 (1.020-2.038)	**0.038**	0.892 (0.600-1.325)	0.571
Sex				
Male	Reference			
Female	1.051 (0.732-1.509)	0.788		
Diabetes				
No	Reference			
Yes	0.900 (0.629-1.286)	0.563		
Liver metastases				
No	Reference			
Yes	1.274 (0.874-1.856)	0.208		
Other metastases				
No	Reference			
Yes	1.541 (1.085-2.187)	**0.016**	0.673 (0.374-1.212)	0.187
Number of organ metastases				
0	Reference			
1	0.817 (0.574-1.164)	0.263	1.382 (0.745-2.565)	0.305

(Continued)

TABLE 3 Continued

Variables	Univariate analysis		Multivariate analysis	
	Hazard Ratio(95%CI)	P value	Hazard Ratio(95%CI)	P value
Number of organ metastases				
≥2	1.921 (1.310-2.817)	**<0.001**	2.499 (1.027-6.080)	**0.043**
Primary site				
Head of pancreas	Reference			
Neck of pancreas	0.920 (0.375-2.257)	0.855		
Body of pancreas	0.922 (0.591-1.438)	0.721		
Tail of pancreas	1.103 (0.721-1.687)	0.653		
Overlapping lesion of pancreas	1.345 (0.930-1.946)	0.116		
Tumor size				
≤52 mm	Reference			
>52 mm	1.656 (1.133-2.418)	**0.009**	1.584 (1.068-2.351)	**0.022**
Chemotherapy				
No	Reference			
Yes	0.542 (0.383-0.766)	**<0.001**	0.569 (0.384-0.843)	**0.005**
Radiation				
No	Reference			
Yes	0.730 (0.393-1.356)	0.319		
Immunotherapy				
No	Reference			
Yes	0.725 (0.434-1.208)	0.217		
Targeted therapy				
No	Reference			
Yes	0.457 (0.261-0.799)	**0.006**	0.478 (0.267-0.855)	**0.013**
LDH				
≤264 U/L	Reference			
>264 U/L	2.080 (1.435-3.016)	**<0.001**	1.359 (0.910-2.031)	0.134
CA19-9				
≤10.5 U/mL	Reference			
>10.5 U/mL	0.704 (0.426-1.163)	0.17		
NAR				
≤0.10	Reference			
>0.10	2.511 (1.741-3.622)	**<0.001**	1.676 (1.080-2.602)	**0.021**
A-SII score				
0	Reference			
1	1.650 (1.151-2.365)	**0.006**	2.005 (1.298-3.099)	**0.002**
2	3.211 (2.038-5.059)	**<0.001**	3.308 (1.872-5.847)	**<0.001**

Statistical differences are indicated in bold.

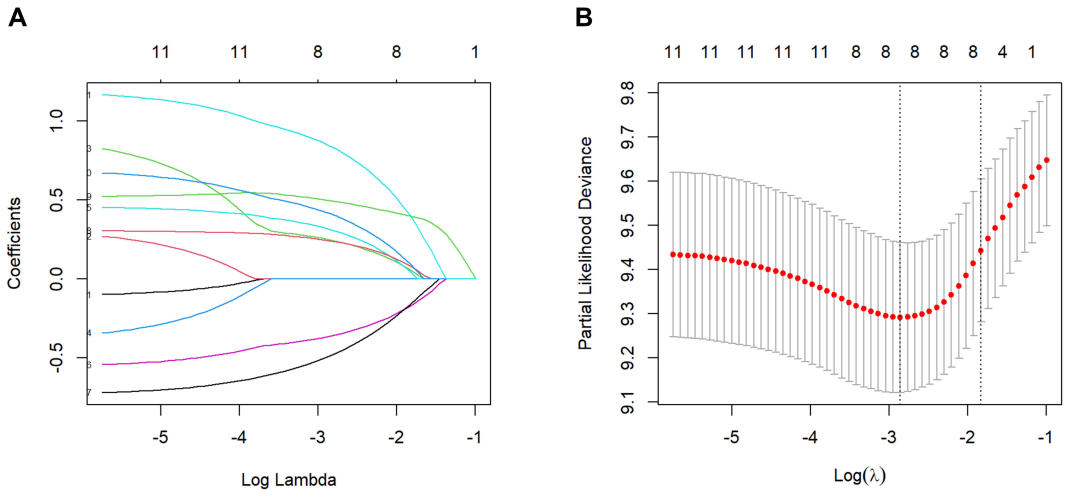

FIGURE 1
Factors selection by the Lasso regression. **(A)** Lasso coefficient profile of the 11 factors. **(B)** 7 prognostic factors were selected based on 1 standard error criteria of the minimum considered as the optimal parameter (lambda) in the Lasso model.

0.774,0.795,0.859 (Figure 2C). ROC analysis of nomogram2 in all enrolled patients showed that AUC of 3-, 6-, and 12-month OS respectively reached 0.796, 0.809, 0.858 (Figure 2D). Considering the C-index and ROC curves in an integrated manner, nomogram2 was superior to nomogram1, so we selected nomogram2 as our visualization model to predict 3-, 6-and 12-month OS.

In addition, the calibration curves of nomogram2 indicated satisfied consistency between actual observation and prediction (Figure 3). To quantify the utility of nomogram2 at specific clinical decision thresholds, DCA curves were plotted, and the result showed that nomogram2 had favorable net clinical benefit (Figure 4).

3.5 Survival analysis

The nomogram2 total points were divided into low risk group and high risk group by the median, and then the K-M survival curves of the training set and validation set revealed a significant difference in survival probability between the two groups ($p < 0.0001$, Figures 5A, B). In addition, as shown in Figure 2B, the A-SII score was the most important factor of all those independent prognostic factors, and we plotted K-M curves of A-SII scores of 0 (n=95), 1 (n=69), and 2 (n=41). The three groups had significant differences in survival probability ($p < 0.0001$), and patients with low scores had better prognoses than those with high scores (Figure 5C). Additionally, a total of 178 patients with mPC were analyzed as a subgroup using K-M survival analysis. In the mPC subgroup, the low risk group had significantly better survival probability than the high risk group, as determined by total points from nomogram2 ($p < 0.0001$, Supplementary Figures S4A, B). K-M curves for patients with mPC, categorized by A-SII scores of 0 (n=82), 1 (n=58), and 2 (n=38), showed significant differences in survival probabilities among the three groups ($p < 0.0001$, Supplementary Figure S4C). Patients with high A-SII scores have significantly worse prognoses than those with low A-SII scores in mPC.

4 Discussion

Since pancreatic carcinoma is hardly diagnosed at the early stages and easily occurs metastases, patients often have progressed to advanced pancreatic carcinoma when diagnosed, and their prognoses are very poor (2, 3). In recent years, immune-inflammatory responses and nutritional status have been found to be relevant to the prognosis of patients with PC, however, the relevant markers of pancreatic carcinoma prognosis are relatively single at present (20, 21). Therefore, in this study, albumin and SII were combined to obtain the A-SII score to make up for the deficiency of a single marker, and a nomogram was constructed on basis of the Lasso Cox regression for predicting the OS of advanced pancreatic carcinoma patients.

Previous studies have shown that the nutritional status and the systemic immune inflammatory response are involved in the occurrence and development of malignant tumors, also influencing the prognoses of patients (17, 18). Serum albumin serves not only as a crucial indicator of nutritional status but also as a significant marker of liver protein synthesis efficiency (22). Previous studies have revealed that tumor cells can produce and release some inflammatory mediators, such as tumor necrosis factor-alpha and interleukin-6, which may inhibit the synthesis of albumin in the liver, potentially resulting in hypoproteinemia (23, 24). Mitsunaga et al. (25)reported that patients with advanced pancreatic carcinoma who exhibited higher levels of IL-6 had lower OS. Additionally, the serum albumin level not only reflects nutritional status but is also associated with the inflammatory response. Cytokines released by inflammatory cells increase microvascular permeability, resulting in greater extravasation of serum albumin through the blood vessel wall (26). Previous studies have demonstrated that serum albumin levels are strongly associated with the prognoses of various malignant tumors, including breast cancer and PC (27). Recently, the ratio of albumin to serum indices, such as C-reactive protein (28) and fibrinogen (29),

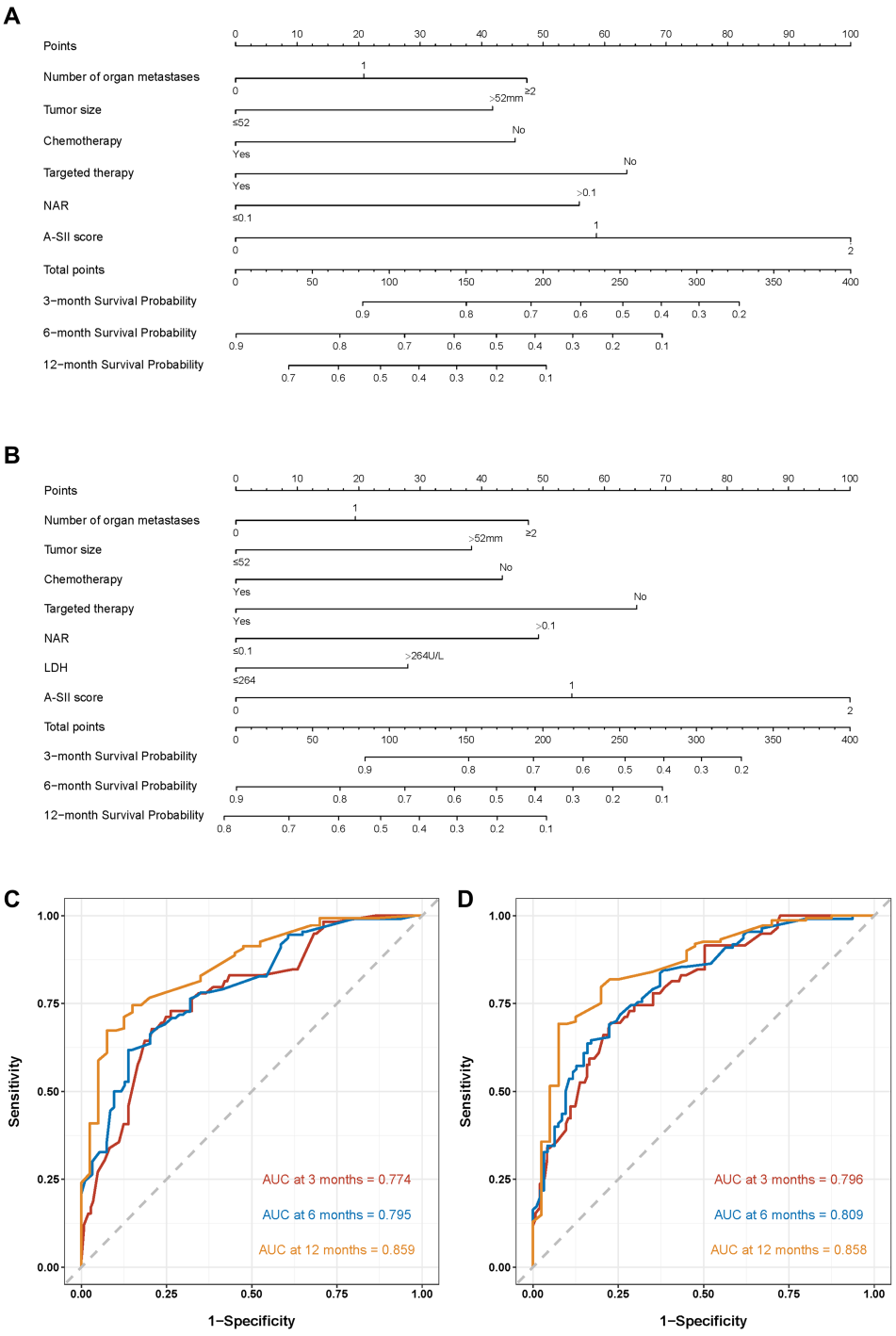

FIGURE 2
Nomograms to predict the probabilities of 3-, 6-, and 12-month OS and their ROC curves. **(A)** The nomogram1 based on the univariate and multivariate Cox regression; **(B)** The nomogram2 on the basis of the Lasso Cox regression. **(C)** ROC curves of nomogram1; **(D)** ROC curves of nomogram2.

has been frequently used to predict the prognosis of PC, providing a reference for constructing the A-SII score in this study. Recently, numerous studies have demonstrated that SII is closely associated with the prognoses of patients with advanced pancreatic carcinoma (30, 31). SII is calculated based on neutrophils, platelets and lymphocytes, all of which are involved in cancer progression. Neutrophils are often recruited into tumor tissues to differentiate into tumor-associated neutrophils and contribute to the formation of tumor microenvironment. Moreover, neutrophil extracellular traps (NETs) generated by neutrophils can enhance tumor cell proliferation and facilitate cancer cell invasion and metastasis (32, 33). Platelets not only release various pro-survival, pro-angiogenic,

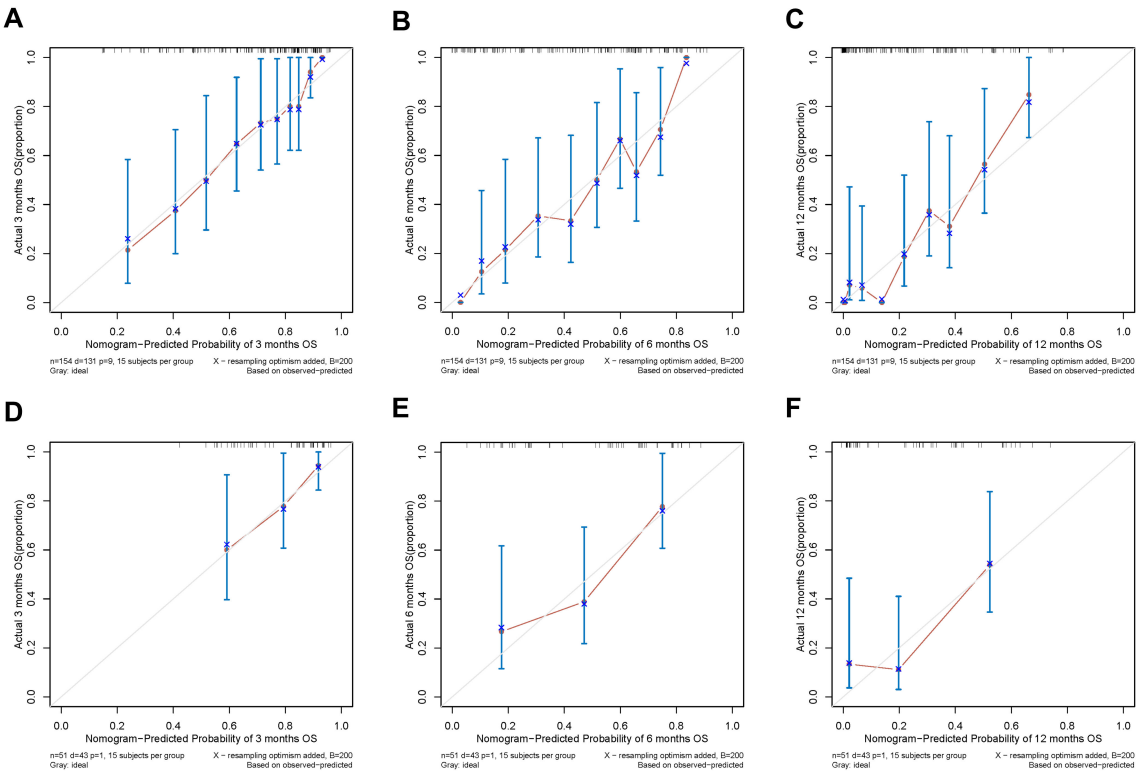

FIGURE 3
Calibration curves of the nomogram2. **(A-C)** the training set; **(D-F)** the validation set.

and immunomodulatory factors to establish and sustain the primary and metastatic tumor microenvironment but also shield tumor cells from immune clearance (34). Conversely, lymphocytes primarily inhibit tumor proliferation and migration while inducing tumor cell apoptosis. The above explanation may provide further clarity on the association between SII and the prognosis of patients with advanced pancreatic cancer. We observed that most previous studies focused on the prognostic value of single indicators, whereas relatively few explored the prognosis of advanced pancreatic carcinoma using integrated indicators of nutritional status, immunity, and inflammation. This study is the first to investigate the prognostic value of albumin combined with SII in patients with advanced pancreatic carcinoma. The t-AUC analysis confirmed that the A-SII score outperformed albumin or SII alone in predicting the prognosis of advanced pancreatic carcinoma. Furthermore, we found that the A-SII score, as one of the independent prognostic factors, contributed the largest contribution to the nomogram. This indicates that combining albumin and SII is essential for the prediction model and assists clinicians in more accurately estimating patient survival probabilities.

The clinical data and follow-up data of 205 patients with advanced pancreatic carcinoma who had not received antitumor therapy were retrospectively analyzed. The multivariate Cox regression and Lasso Cox regression were used to confirm the independent prognostic factors. Through the comprehensive comparison of C-index and AUC values, nomogram2 established by the Lasso Cox regression which identified 7 variables, including the number of organ metastases, tumor size, chemotherapy, targeted therapy, NAR, LDH, A-SII score, is superior to nomogram1 based on the multivariate Cox regression. Patients with advanced pancreatic carcinoma often develop metastases, and our study showed that patients with multiple metastases had poorer prognoses, consistent with Feng et al.'s study (35), which indicated that patients with multiple metastases in metastatic pancreatic carcinoma had lower OS. In a study of 1,898 patients with liver metastases in PC (36), Shi et al. identified tumor size as an independent prognostic variable, similar to our findings. Chemotherapy is the first-line treatment for advanced pancreatic carcinoma, and multiple studies have confirmed that chemotherapy improves clinical outcomes in advanced pancreatic carcinoma (36, 37), findings consistent with ours. However, for patients with advanced pancreatic carcinoma, single chemotherapy does not meet the demands of clinical multimodal therapy. Recent studies have shown that targeted therapy has potential for clinical application as a novel anti-tumor strategy, as certain clinical trials targeting aberrant pathways and molecular abnormalities have yielded promising results. Targeted therapy has become a new anti-tumor approach in clinical practice. Erlotinib combined with selumetinib demonstrates antitumor efficacy in locally advanced or metastatic pancreatic ductal adenocarcinoma (38). IGF-1R antagonist (MK-0646) in combination with gemcitabine can synergistically enhance OS. This study demonstrates that targeted therapy can also improve OS among advanced pancreatic carcinoma (39, 40). Currently, limited research has explored the

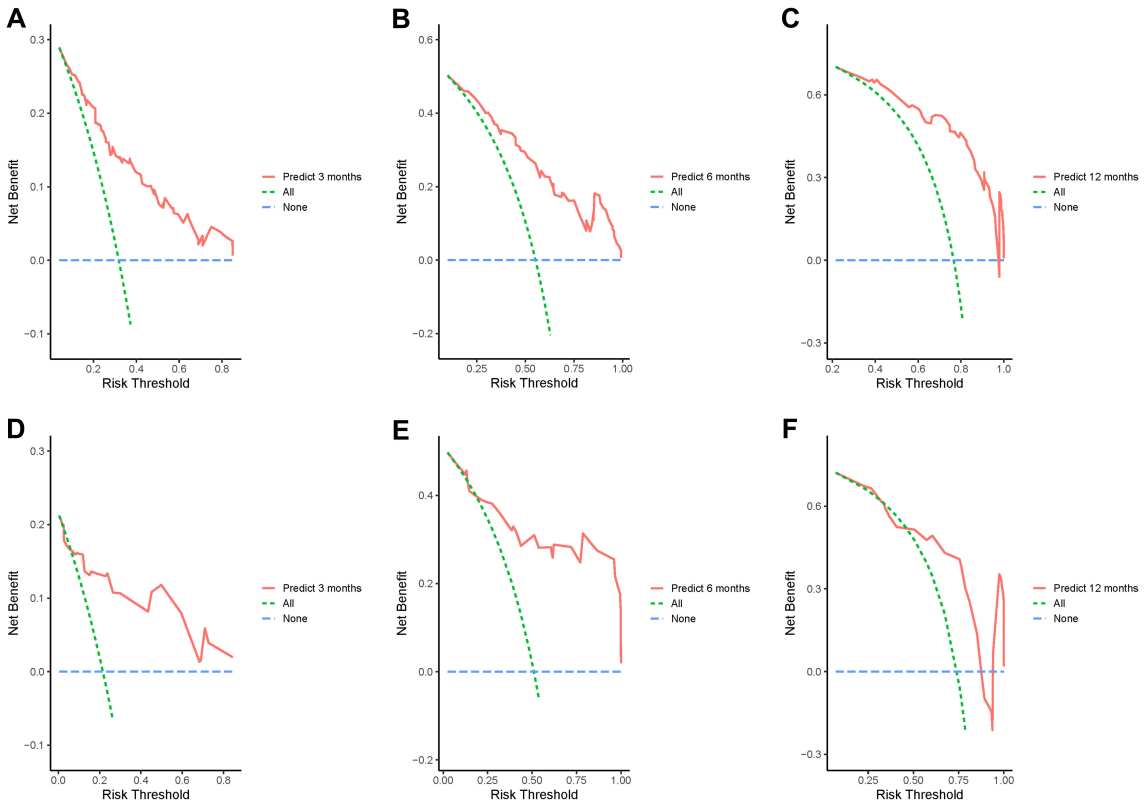

FIGURE 4
Decision curve analysis of the nomogram2. **(A-C)** the training set; **(D-F)** the validation set.

relationship between NAR and the prognosis of advanced pancreatic carcinoma. Tingle et al. reported that NAR combined with CA19-9 could effectively predict OS among patients with palliative pancreatic cancer (41). Our study further validated that NAR is a significant prognostic factor in advanced pancreatic carcinoma. It is well known that tumor cells primarily depend on anaerobic glycolysis for energy production, and LDH, a key enzyme in tumor cell metabolism, plays a crucial role. Our results indicate that elevated LDH levels are linked to poor prognosis in advanced pancreatic carcinoma. Similar findings have been observed in non-small cell lung cancer and colorectal cancer (42, 43).

Although CA19-9 has long been considered to play an important role in the diagnosis and prognosis of PC, this study determined that CA19-9 is not an independent prognostic factor in advanced pancreatic carcinoma. Our analysis may be related to the following reasons (1): 5%-10% of patients lack the Lewis blood group antigens, leading to negative CA19-9 results (44); (2) Obstructive jaundice caused by pancreatic head cancer may lead

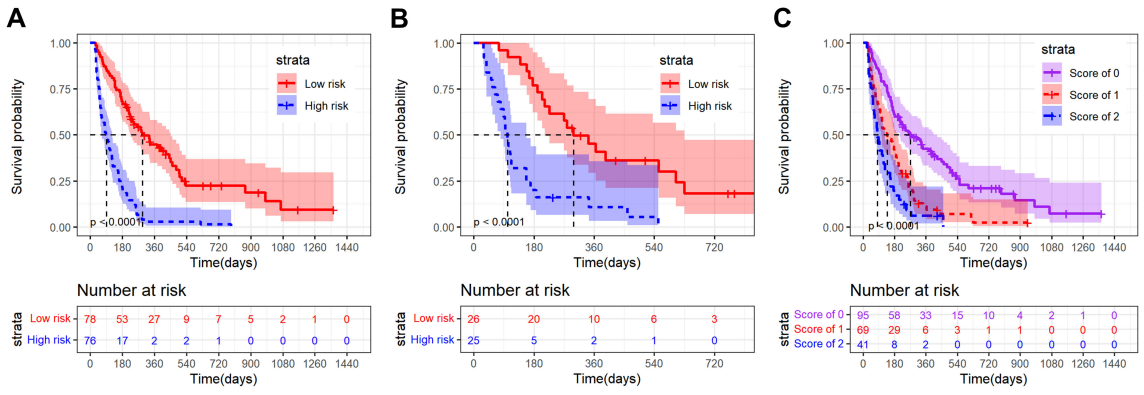

FIGURE 5
Kaplan-Meier survival curves of advanced pancreatic carcinoma patients divided into different strata according to the nomogram2 total points or A-SII score. **(A)** K-M curve of the training set in low risk and high risk groups on the basis of the nomogram2 total points; **(B)** K-M curve of the validation set in low risk and high risk groups on the basis of the nomogram2 total points; **(C)** K-M curve of all patients in the A-SII score of 0,1 and 2 groups.

to elevated CA19-9 levels; (3) Studies have shown that dynamic changes in CA19-9 levels may better facilitate the evaluation of PC prognosis (37). In the phase 3 Metastatic Pancreatic Adenocarcinoma Clinical Trial, CA19-9 levels were not significantly associated with the prognosis of metastatic pancreatic adenocarcinoma in multivariate Cox regression analysis (45). Moreover, in exploring the prognostic value of NLR in mPC, a finding found that CA19-9 levels were not correlated with OS (46), which is consistent with ours. In contrast, some studies have shown that CA19-9 levels are strongly correlated with OS in advanced pancreatic carcinoma (47, 48). Given the contradictory evidence regarding the prognostic value of CA19-9, additional robust clinical studies are required to confirm these findings beyond the factors analyzed in our study.

At present, Lasso Cox regression is widely used in gene screening (49), but recently several studies have shown that Lasso Cox regression has important value in screening clinical indicators and constructing predictive models. Zhou D et al. used Lasso Cox regression to establish a nomogram of alpha-fetoprotein-negative hepatocellular carcinoma patients without surgery (50). Fan X et al. established a nomogram based on Lasso Cox regression for predicting OS of patients with T1b esophageal cancer treated by endoscopy (51). However, Lasso Cox regression is rarely used in PC. In order to eliminate the problem of multicollinearity between variables, this study constructed the nomogram2 on the basis of Lasso Cox regression to predict the 3-, 6-, and 12-month OS of patients with advanced pancreatic carcinoma, which showed better prediction performance than the nomogram1 on the basis of multivariate Cox regression. The calibration curve and DCA curve show that nomogram2 has good accuracy and clinical net benefit.

Although this study proposed for the first time that the A-SII score is a crucial independent prognostic factor for advanced pancreatic carcinoma, it still has its limitations. Firstly, the study was retrospective and restricted by the number of samples, which may cause selection bias. Secondly, it was a single-center study that lacked a corresponding external validation set. We look forward to conducting large sample studies to further confirm the current conclusions in the near future.

5 Conclusion

A-SII score is a crucial independent prognostic factor for patients with advanced pancreatic carcinoma. In this study, a nomogram based on Lasso Cox regression was established for predicting advanced pancreatic cancer patients 3-,6- and 12-month OS, which has good predictive ability and could accurately distinguish low risk and high risk groups of advanced pancreatic carcinoma.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by Ethics Committee of the Affiliated Yancheng First Hospital of Nanjing University Medical School. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements.

Author contributions

MX: Writing – original draft, Writing – review & editing, Data curation, Methodology. YL: Writing – original draft, Writing – review & editing. PC: Validation, Writing – original draft. AL: Conceptualization, Writing – original draft. JX: Data curation, Writing – original draft. YX: Supervision, Writing – review & editing, Writing – original draft.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2025.1447055/full#supplementary-material

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